VOLUME XXIII NUMBER 6

BOTANICAL GAZETTE

JUNE 1897

CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XXXIX.

FURTHER OBSERVATIONS ON THE MYXOBACTERIACEÆ.

ROLAND THAXTER.

(WITH PLATES XXX-XXXI)

IN a paper published in the GAZETTE[1] a few years since the writer presented certain notes concerning a curious group of Schizomycetes, which, from the fact that their life cycle is divided into two definitely recurring periods—one of vegetation, the other a period of fructification or pseudo-fructification through the simultaneous and concerted action of numerous individuals—was thought to be sufficiently well characterized to warrant the separation of its members as a distinct order. The name Myxobacteriaceæ was selected as an appropriate designation for them, in view of the very striking similarities which become evident when one compares the life cycle just mentioned with that of the Mycetozoa; the general characteristics of the two periods being, in either case, practically identical, except for the differences presented by the cell structure of the individual organisms concerned. Since the publication of the paper referred to, the remarkable nature of this resemblance has been further enforced through the appearance of a recent paper by Dr. Zukal[2], in which, under the title "Myxobo-

[1] BOT. GAZ. **14**: 389. 1892.

[2] Berichte d. d. bot. Gesell. **14**: 340. 1896.

trys variabilis als Repräsentant einer neuen Myxomyceten Ordnung," he gives an account of a singular plant, at least very like *Chondromyces crocatus* of my former paper, the characters of which are in his opinion so similar to those of the Mycetozoa that he does not hesitate to place the organism in question near Ceratiomyxa among the "Exosporeae." The vegetative mass of Myxobotrys is held to be a true plasmodium which is said to ingest its food like the plasmodium of a myxomycete, and in a similar fashion rids itself of all foreign matters before rising to form its fructification; the gelatinous matrix about the rods being looked upon as hyaloplasm; while the rod-like structures are described as "microsomata" imbedded in it. It is further stated that, as the plasmodium rises to fructify, the microsomata suddenly disappear and are replaced by numerous long cylindrical filaments, and that these same filaments fill the "spores" at maturity, winding about in their interior.

In a more recent "Notiz zu meiner Mittheilung über Myxobotrys variabilis" Dr. Zukal calls attention to the identity of this species with *Chondromyces crocatus*, making further suggestions as to its probable synonymy which will be mentioned below. He further remarks that, strange as it may appear, the present writer's view as to the schizomycetous nature of this organism is worthy serious consideration, although he further asserts his own disbelief in the truth of this assumption, as well as his adherence to the opinion expressed in his first paper, namely, that the organism in question belongs not to the bacteria, but to the Mycetozoa. In view of the striking and important differences which would seem to distinguish Myxobotrys, as described by Dr. Zukal, from any member of the Myxobacteriaceæ with which I am familiar, it is perhaps not altogether safe to assume its identity with *Chondromyces crocatus*, although the two appear to approach so closely in general habit. If, however, we assume this identity and admit for the moment the correctness of Zukal's observations, it must be confessed that his interpretation of the development described by him is quite as remarkable as the facts themselves. A true plasmo-

dium, consisting of a matrix of hyaloplasm wholly destitute of nuclei and apparently of any structure whatever, in which are embedded rod-like microsomata capable of entering upon an independent existence when separated from the plasmodium, as well as possessed of a Beggiatoa-like power of locomotion and a capacity for indefinite multiplication by fission, coupled with a faculty of transforming themselves suddenly into slender filaments, would be sufficiently singular, even were not these same "microsomata" demonstrably cells, similar to the cells of other schizomycetes; and did not these cells, like other bacteria, pro duce, in a whole series of forms, definitely differentiated spores capable of germination.

That all plasmodia, as suggested by further observations recorded in the "Notiz" just mentioned, are likely to prove similar to the vegetative mass of the Myxobacteriaceæ, and that they will be found to possess a mode of increase, hitherto overlooked, by means of bacteria-like energids, seems unlikely in view of the well known fact that the energids of true plasmodia are potential amœbæ. It is hardly necessary to remark that I am unable to agree with Dr. Zukal in considering the Myxobacteriaceæ members of the Mycetozoa; and further observation of them during the past five years, during which certain species, including *C. crocatus*, have been kept in constant cultivation in my laboratory, has served fully to confirm the correctness of the views previously published in the GAZETTE. Having been occupied during this time with other matters, I have been unable to pay special attention to the group; yet have succeeded in accumulating a certain amount of new material, and have made a few additional observations in connection with the development of its members, the most important of which relate to the sporulation and spore germination of the species of Myxococcus; both which matters were left undetermined in my previous paper. The present note is therefore offered as a further contribution towards a knowledge of the species, as well as of the development of a group of organisms which, as is evidenced by its entire omission from a work like the *Pflanzenfamilien* of Engler

and Prantl, does not appear to have received the recognition it well merits. That the family, as predicted in my former paper, is likely to prove by no means numerically insignificant, is indicated by the additions enumerated below, which have for the most part made their appearance by accident, as it were, on laboratory cultures designed for other purposes; and it seems probable that any systematic attempt to enlarge our knowledge of the species could hardly fail to produce interesting results.[3]

It may be remembered that, in arranging the nine species which formed the nucleus of the order, two sub-groups were distinguished; the one including in the single genus Myxococcus all those forms in which the individuals become transformed into definite spores during the period of fructification; while all the remaining forms were included in the two genera Chondromyces and Myxobacter; the individuals in both these instances becoming encysted *en masse*, without being converted into definitely formed spores. In the first category those species in which the spore mass is permanently encysted or definitely coherent at maturity were further distinguished from those in which it soon becomes deliquescent, while in the second category those forms which produce their cysts free in the air (Chondromyces) were separated from those in which the latter are formed embedded in a gelatinous matrix, the two species included in the last mentioned class being placed in the genus Myxobacter, which, as will be presently seen, may be referred with little doubt to Schroeter's Cystobacter.

Although all of the forms described below fall under one or the other of the above categories and no new generic types are included among them, the gross structure of the fructifying condition in several of them presents points of no little interest. In

[3] Since the present paper was in press Messrs. Pound and Clements in their "Rearrangement" of the North American Hyphomycetes (*Minnesota Botanical Studies*, 44) have referred the lichenicolous species of Illosporium to the Myxobacteriaceæ. The species of this genus, however, are semi-sclerotic conditions of hyphomycetous fungi, which, in some instances at least, are connected with species of Nectria, and in no case can be mistaken for Myxobacteriae. Why these authors retain the genus Stigmatella in their rearrangement is not apparent.

the Myxococcus group, for example, the species previously enumerated were all characterized by a practically sessile habit, and in the absence of a knowledge of their complete life history their close relationship to the more highly developed cystophore-producing forms might well have been questioned. The discovery, however, of the species subsequently described as *Myxococcus stipitatus* serves more definitely to indicate the close relationship between the two groups, since in this instance the spore mass is raised above the substratum on a well developed stalk, corresponding exactly in structure and method of formation to the often highly differentiated cystophores characteristic of nearly all the species of Chondromyces; the only difference being dependent on the fact that in the one case the ultimate mass or masses of individuals become encysted as such, while in the other they form an eventually deliquescent spore mass. A further indication of this relationship is also found in the characters which distinguish *Myxococcus cruentus*, the cysts in this species being very clearly differentiated, with a well defined wall surrounding the mass of spores within, which are themselves embedded in a stringy, coherent though scanty matrix that recalls the corresponding condition found in the cysts of Chondromyces. The spores, moreover, are not as well developed as in the other species of Myxococcus, and seem to suggest a transitional form between a slightly modified rod, such as occurs in the cysts of Chondromyces, and a typical spore like that which is found, for example, in *Myxococcus rubescens*. The general habit of the species closely resembles that of Cystobacter as below emended; and, were it not for the definite spores, might readily be included in that genus. In this connection it may be mentioned, moreover, that an examination of the contents of mature cysts of *Cystobacter fuscus* shows that the rods in this case are more definitely modified than in the species of Chondromyces; their walls being visibly thicker and their contents enclosing the same definitely formed nucleus-like body described below as characteristic of the developing spores of Myxococcus. It should be mentioned, however, that I have not yet had an opportunity of

reexamining fresh material of the other species of Myxobacter, in order to ascertain whether they exhibit similar modifications.

The most important fact, which further examination of the sporiferous forms has served to determine, is connected with the process of spore formation and germination, matters which in my former paper were left in doubt from lack of exact observations; certain appearances which one often sees in the rising masses of spores, having, in the first instance, led to the erroneous suggestion there made that they were produced in chains, while the process of their germination was also left undetermined. Further and more extended examination of these processes in pure cultures of *Myxococcus rubescens* shows that my previous conclusions in regard to these matters are not in accordance with the facts, since the phenomena in either case prove to be such as would naturally be associated with the development of organisms of this nature.

As was mentioned in my previous account, sporulation appears invariably to take place gregariously, if one may use such an expression to indicate that isolated rods have never been seen to become thus transformed. The impulse to sporulate thus seems to be, as it were, contagious, and takes possession of a large number of rods simultaneously, which, in their turn, exercise a similar influence on other rods in their more immediate vicinity; so that a condition of things exists at this period which serves still further to accentuate the remarkable correspondence between the Myxobacteriaceæ and the Sorophoreae or pseudoplasmodium-forming Mycetozoa. From the fact, therefore, that sporulation only occurs in the rising rod mass, and that it takes place more or less continuously, in a narrow zone only, below the spore mass, which for a certain period is thus constantly being augmented from below, direct observation of the process, as for example in Van Tieghem cells, is almost impossible. It is therefore only by removing from pure cultures and crushing whole guttulae that the successive stages can be obtained. Both rods and spores are in general so minute and become so promiscuously intermingled by this treatment that,

except in very favorable instances, the true nature of the process is not at once apparent, although, when once ascertained, it has been readily demonstrated in all the species examined. By removing and crushing guttulae of *Myxococcus rubescens*, for example, which have formed on agar in pure cultures, selecting preferably such as have recently begun to rise from the substratum, it is not difficult to obtain in abundance all stages illustrating the process of sporulation; and by staining the material with Delafield's haematoxylin, or with eosin, the mature spores which resist stains are at once differentiated in the preparation from the immature conditions. It may be thus definitely determined that sporulation consists in the direct transformation of single rods into single spores. By a gradual inflation, involving a corresponding shortening of the rod in the direction of its long axis, even the longest individuals eventually assume a spherical form, and through the deposition of material on the inner surface of their walls are gradually converted into thick-walled refractive spores. The successive steps in this transformation may be more readily understood by reference to *fig. 36* (*a–j*), and it may be mentioned that, as is indicated in the figures, a marked enlargement is first apparent at one end of the rod, the progressive transformation gradually involving the whole cell. As in other members of the group, the rods, when they run together to sporulate or to produce cysts, become thickened and somewhat shortened, and the more densely granular portion of their contents tends to collect in somewhat definite masses. Even before the rods have begun to show the terminal inflation just mentioned, one of these masses towards the extremity of the cell may be seen to be distinctly larger than the rest, which tend to disappear as the transformation of the rod progresses; the larger mass becoming more and more distinct and clearly defined, taking a deep stain with either eosin or haematoxylin. As the spore assumes a more rounded form this deeply staining mass comes to occupy a central position, and has the appearance of a well defined nucleus-like body (*h* and *i*), which continues to stain readily and deeply until the wall

becomes so thick that the whole spore remains bright and refractive and impenetrable by stains, except after they have acted for a considerable time.

The occurrence of this nucleus-like body in the spores of Myxococcus led me to examine more carefully the conditions found in the rods of Chondromyces while becoming encysted, and in these instances also the granular more deeply staining portions of the cell contents were found to collect in more or less definitely circumscribed masses. In Chondromyces this mass, though more frequently single, was found to be generally elongate, occupying a central or usually somewhat lateral position (*fig. 15*). In the rods which compose the ascending "pseudoplasmodium" in this genus, the granular masses are very readily demonstrated by staining, and have in general the appearance represented in *fig. 14*, a small mass being commonly distinguishable at either end of the cell, while a much larger and more irregular one is apt to occupy a central position. In *Cystobacter fuscus*, on the other hand, a portion of the more deeply staining contents, as has been previously mentioned, was found to become definitely aggregated into a well defined nucleus-like body corresponding exactly in its general appearance to that of the sporulating forms just described, an accompanying thickening of the wall being in many cases distinctly visible.

Such transitions between a slight and a complete transformation in the rods at the period of fructification are doubtless correlated with an inverse differentiation of the cyst, the most perfectly formed spores being those characteristic of deliquescent guttulae, while the least well marked differentation of the individual rods occurs in forms like *Chondromyces crocatus* in which the cysts reach a maximum development.

At the time when my first notes were published no satisfactory data had been obtained in regard to the germination of the spores in any case. In cultures of Myxococcus made from material which had been kept air dry for several months, the spores when placed in a nutrient medium gradually assumed a short stout rod form; but no separation of this body from the

spore wall was noticed nor was any further development of the rods by fission seen, although both may have been overlooked. Recently, however, by cultivating in Van Tieghem cells the spores of *M. rubescens* taken from pure agar cultures, I have repeatedly obtained abundant germinations and have been able to follow the whole process with a $\frac{1}{16}$ oil immersion. The most convenient mode of procedure for this purpose I have found to be as follows. A small amount of the spore-material, which is easily obtained free from rods when taken from pure agar cultures, was spread near the center of a sterilized cover glass; and when thoroughly dried on, so that the spores adhered firmly, was covered with a thin slice of nutrient agar transferred directly from the surface of a sterilized agar tube. The coverglass was then mounted on the cell in the ordinary fashion, a small amount of water containing unicellular algæ being added within it to furnish moisture and oxygen. The spores were thus firmly fixed in definite positions on the under side of the cover and could be readily examined directly with the immersion objective. In these cultures the germinations began to be visible in from one to two weeks, seven days being the shortest period within which they occurred; and in all cases they were of a single type, no instances being noticed in which the spores underwent the gradual change of form above described, which on the assumption that no separation of the rod from the spore wall occurred, must, I think, be considered an abnormal phenomenon.

The first indication of germination, as shown by preparations which, since they could not otherwise be stained, were made directly from the Van Tieghem-cell cultures, consists in the slight enlargement of the spore and the recovery of its power quickly to absorb stains. Such deeply stained spores are conspicuous in a field, contrasting with the still refractive and wholly colorless ones in which germination has not yet commenced. The walls of the spores thus stained (*fig. 34*) appear as if irregularly corroded by absorption from within, and eventually become comparatively thin, often more so at one or two definite points than elsewhere. At such a thin point a protrusion is

then seen, which gradually increases, and at the same time the outline of a stout rod, sometimes bent or irregularly swollen, becomes more and more distinct within the spore-wall (*fig. 35*). The emerging rod elongates with considerable rapidity, until it becomes about twice as long as the diameter of the spore. In several cases it was then seen to slip out free from the spore wall (*fig. 35, h*, and, four minutes later, *i*) which remains for a time as an empty shell, but gradually dissolves and disappears. More commonly, however, the emerging rod does not escape from the spore wall, but remains attached to the latter, being apparently fixed within it, elongating and dividing as is indicated in the series of figures (*c–g*) which represent the successive stages in the development of such a rod. In very many cases the emerging rod may be seen, as in *j* and *k*, to have penetrated the spore wall on both sides, so that two free ends project and continue to grow and divide as usual till the old spore wall disappears through absorption. That such appearances do not represent accidental superpositions, I have been able to determine to my own satisfaction.

The addition of the following species, the number of which might be augmented by others that from lack of proper material I have been unwilling to publish, more than doubles the number of representatives of the group formerly enumerated and serves to enforce with even greater emphasis than was before possible the fact that the course of development of these organisms forms a distinct departure from that of other Schizomycetes, in that their life cycle, as has been pointed out, is divided into two distinct periods; the one of vegetation, the other of fructification or pseudo-fructification resulting from the concerted action of many independent individuals toward this very definite end. This view is here reiterated for the reason that in one of the few references to the group that have come to my notice since the publication of my first paper, it is held that the aerial character of the cysts, and not the circumstance just mentioned, should be regarded as the crucial point of difference between the Myxobacteriaceæ and other Schizomycetes. As a

matter of fact, the cysts or spore masses are not always produced vertically free from the substratum; and were forms discovered, as they well may be, which reached maturity in water or embedded in the nutrient matrix, they would still be clearly distinguished as above indicated.

Among the forms described below belonging to the genus Chondromyces the most striking is that which I have called *C. apiculatus*, an African species distinguished from its ally, *C. crocatus*, by a curiously well defined and constant specific character, which, though quite as reliable as a well marked difference in spore-form would be in the higher fungi, is here dependent on a slight variation in the movements of the individuals composing the ultimate rod masses during the maturation of the cysts, the invariable recurrence of which gives to the mature cysts a specific form. This form results from the fact that the cyst, when it has received all the rods destined for it, becomes at first fusiform, as in *C. crocatus*, the extremities, however, being more attenuated than in this species; but while in *C. crocatus* the mass of rods composing it, together with the film of hardened gelatinous material which has been secreted around them, gradually assumes the subconical shape characteristic of the species, the individuals in the cyst of *C. apiculatus* migrate from each extremity towards the center, this movement taking place within, and without involving the surrounding membrane which is thus left empty at each end, the empty portions corresponding to the shrivelled appendages which distinguish the mature cysts.

Among the other species the most interesting are perhaps the *Cystobacter erectus* and *C. fuscus* of Schroeter. Of these the former (*figs. 16–19*), although undoubtedly a Chondromyces, proves quite distinct from *C. aurantiacus* with which I was formerly inclined to unite it; while the latter, since it is doubtless congeneric with the species of my own genus Myxobacter, may be considered as the type of the genus Cystobacter, a name which, of course, antedates my own.

Chondromyces apiculatus, nov. sp. *Plate XXX, figs. 1–15.*—Cystophore stiff, rigid, simple, rarely sparingly branched,

bearing the single spherical cyst-mass terminally. Cysts very variable in form, shape and size, cylindrical to broadly turnip-shaped, the young cysts fusiform or nearly so, the rods retreating from each end towards the center and leaving behind a shriveled membrane forming a basal and terminal appendage, the latter longer and pointed. Color bright orange. Rods 1 by 3–20μ, sometimes longer. Head of cysts 136μ, average about 200μ. Cysts, turnip-shaped form, average about 35μ broad by 28μ long; cylindrical form, average 35 by 18μ. Cystophores about 500–1000μ in height. All dimensions subject to great variations.

On dung of antelope from Liberia, Africa.

Two or three cystophores of this striking species made their appearance on some antelope dung sent me from Africa, which I owe to the kindness of Mr. F. C. Straub. Having been successful in propagating it from these original specimens I have kept it in constant cultivation for more than a year, and have thus been able to determine the constancy of the characters which separate it from its nearest ally, *C. crocatus*. Its general color is somewhat more orange and less yellow than that of the last mentioned species, and though rarely branched it never develops the highly differentiated cystophores so characteristic of this species when growing under favorable conditions. The head is almost invariably solitary under the most favorable conditions, and the mature cysts are always characterized by the peculiar shrivelled appendages to which reference has already been made above. The variations in the form of the cysts is very great, the most typical and striking having the turnip or onion-shape represented in *figs. 5, 10, 11*. The cysts not infrequently fuse laterally while in process of formation so that conditions similar to those represented in *figs. 8, 12* are sometimes found, which have resulted from the fusion of two and three cysts, respectively. The germination of the cysts is as readily observed as in *C. crocatus*, but, unlike that species, takes place not only normally at the base, but also at the apex, as is represented in *fig. 13*. The species never grows as readily or as luxuriantly as *C. crocatus*, and I have found great difficulty in inducing it to grow pure on nutrient agar, on which it develops very slowly, and seldom produces cysts and cystophores.

Chondromyces gracilipes, nov. sp. *Plate XXXI, figs. 20–24.*—Color orange red. Cystophore simple, tapering distally to a pointed apex, rigid and persistent on the substratum. Cysts solitary, terminal, oblong to oval, rounded distally, somewhat

flattened basally, caducous. Rods minute, slender, 0.6 by 2–5μ. Cystophore 25–40μ high. Cysts average about 25 by 35μ.

On rabbit dung, Arlington, Mass.

This minute and well marked species made its appearance on a laboratory culture of rabbit dung which it covers with a powdery orange coating. It appears to be constant in its characters, and is abundantly distinct from any of the other known species of the genus.

Chondromyces erectus (Schroeter). *Plate XXXI, figs. 16–19.*

Cystobacter erectus Schroeter, Kryptogamenfl. v. Schlesien, III Band, 1 Lief. p. 170.

Color orange red turning to chestnut brown. Cystophores fascicled, united at the base in groups, above simple or sparingly branched, bearing a single terminal broadly oblong or rounded cyst on a broad base. Cystophores withering at maturity so that the cysts often appear sessile. Rods 0.9 by 2–5μ or longer. Cysts average about 50 by 40 μ. Cystophore 60–300 μ or more in height.

On horse dung in laboratory cultures. Cambridge, Mass.

This species has appeared repeatedly in laboratory cultures within the past few years and seems abundantly distinct from its nearest ally *C. aurantiacus*, its fascicled habit, single terminal cysts, and the chestnut brown color of the latter when mature serving to distinguish it readily from any of the varieties of *C. aurantiacus* with which I am familiar. The cysts of the latter sometimes become brown with age, but in the present instance they assume this color as soon as they are mature.

CHONDROMYCES AURANTIACUS (Berk. & Curtis) Thaxter.

In my former paper on the Myxobacteriaceæ it was suggested that this species was probably synonymous with *Stilbum rhytidospora* figured by Berkeley and Broome in their Fungi of Ceylon, as well as with the *Polycephalum aurantiacum* of Kalchbrenner and Cooke described from African specimens. Although this suggestion was based only on an examination of the figure of the former and description of the latter, it proves to have been correct according to the statement of Massee,[4] who has examined the types of these two forms, as well as that of *Stigmatella aurantiaca*. The synonymy formerly given with a query may therefore be considered correct. The species has been found commonly in many localities and has been sent me from Ohio by Professor Morgan. It has also made its appearance in abundance and in the

[4] Grevillea 21 : 124.

typical form on the same antelope dung from Africa which yielded *C. apiculatus*, and under conditions that made its African origin unquestionable. The species is totally distinct from *C. crocatus*, with which Dr. Zukal would unite it, both in the form and character of its cysts and cystophores, as well as in its color, and is in general very constant, although what appears to be a variety of the same form accompanied the Liberian material and differs from the fact that it is larger and often copiously branched, one "individual" thus producing a number of heads.

CYSTOBACTER FUSCUS Schroeter. *Plate XXXI, figs. 37–39.*

Schroeter Kryptogamenfl. v. Schlesien, III Band. 1 Lief. p. 170.

Color orange red becoming chestnut brown, the rising rod masses pale flesh colored. Cysts formed by the separation of the parts of a more or less convoluted rod mass, nearly spherical to long-oblong or irregularly elongated at maturity, surrounded by a gelatinous matrix, heaped together or lying in one plane on the substratum, each cyst surrounded by a thin, papery, separable chestnut-brown wall; when dry dark dull red. Rods slender, elongate, 0.6 by 5–12 μ. Cysts 50–150 by 50–70 μ.

On dung of rabbits from southern California.

This interesting form made its appearance in abundance, together with *Myxococcus coralloides*, *Pilaira Cesatii*, and several other interesting plants, on rabbit dung from southern California, for which I am indebted to Mr. F. H. Billings. It is a conspicuous species, growing and producing its cysts readily on agar, and seems to correspond in all essentials to the generic type which I formerly called Myxobacter from the fact that its cysts are embedded at maturity in a mucus envelope. Its characters seem to be so nearly identical with those of Schroeter's species that I have no hesitation in referring it to *C. fuscus*, which, it may be remarked, was also found on the same substratum. Assuming that this reference is correct, Myxobacter must be superseded by the earlier name, under which should be included *Cystobacter aureus* and *C. simplex*.

Myxococcus stipitatus, nov. sp. *Plate XXXI, figs. 30–33.*

Color white to pink or flesh color. Spore mass becoming deliquescent, subspherical, formed at the apex of a well developed stout stalk which raises it free above the substratum. Rods 0.5–07 by 2–7 μ or longer. Spores oval, 0.8–1.2 by 1–1.15 μ. Spore mass about 175 μ in diameter. Stalk 100–200 by 30–50 μ.

Dung of sheep, pig, and other animals. Cambridge, Mass.; Kittery Point, Maine; Burbank, Tennessee.

This striking form has made its appearance not infrequently on laboratory cultures and grows luxuriantly on nutrient agar, although it does not fructify on this substratum as readily as the sessile species. The form obtained on pig dung at Burbank, Tenn., was distinctly smaller in habit and milky white in color; but cultures of this variety on agar reverted to the ordinary pinkish form from which it can hardly be distinct. As already mentioned, the stalk is formed in the same way that the cystophores of Chondromyces are produced, and is persistent after the spores have separated from it.

Myxococcus cirrhosus, nov. sp. *Plate XXXI, figs. 25–27.*

Color pale reddish or flesh colored. Spore mass more or less elongate, erect, the base slightly swollen, the distal portion tapering to a rounded apex. Spore irregularly spherical, about 1 μ in diameter. Rods 0.8 by 2–5 μ or longer. Spore mass 50–100 μ high, about 20 μ in diameter at the base.

On grouse dung from Readville, Mass.

This form appeared on a laboratory culture and is so minute and inconspicuous from its pale color that it is seen with difficulty, the more so since the bases of the spore masses are usually more or less embedded in the substratum. The spores although somewhat loosely coherent at maturity do not form a deliquescent mass, so that the species is evidently allied to the section of the genus which includes *M. coralloides.*

Myxococcus cruentus, nov. sp. *Plate XXXI, figs. 28–29.*

Color deep blood red. Cysts regularly spherical, surrounded by a more or less well defined rind or wall within which the spores are embedded in a scanty and amorphous matrix. Rods 0.8 by 3–8 μ. Spores oval or irregularly oblong, 0.9–1 by 1.2–1.4 μ Cysts 90–125 μ in diameter.

On cow dung, Burbank, Tennessee.

This species was found in woods covering the substratum with a blood red coating resembling some dark red Nectria. The cysts are densely aggregated, remarkably uniform in shape and size, and are peculiar from the presence of a moderately well defined wall to which attention has been called above. The spores are more than usually irregular in size and form, and are less well defined than in the other species, resembling in some respects the thickened individuals which occur in the cysts of Chondromyces. The species was not cultivated, and no satisfactory material of its vegetative condition was obtained.

CAMBRIDGE, MASS.

EXPLANATION OF PLATES XXX AND XXXI.

The figures are from ink drawings reduced about one-fourth by photolithography. The letters and numbers refer to the Zeiss or Leitz objectives and eyepieces used in making the original drawings. The approximate magnifications in diameters of these combinations, allowing for projection, are (in the original) as follows: A, oc. 4, 180: C, oc. 2, 230: C, oc. 4, 400: D oc. 2, 380: D, oc. 4, 700: $\frac{1}{16}$ (oil), oc. 4, 1900: $\frac{1}{16}$ (oil), oc. 12, 3300.

Chondromyces apiculatus Thaxter.

FIG. 1. Rising rod mass. A 4.

FIG. 2. Branching cystophore, the cysts just beginning to bud out from right head. A 4.

FIGS. 3–4. Typical cystophores with cysts in different stages of development. A 4.

FIG. 5. Cystophore bearing mature cysts of the turnip-shaped type. A 4.

FIG. 6. Young cyst before the rods have retreated from either end. D 4.

FIGS. 7–12. Mature or nearly mature cysts, *figs. 10, 11* of the turnip form, the rest of the subcylindrical type; *fig. 8* showing the union of two and *fig. 12* of three cysts by lateral fusion. All D 4.

FIG. 13. Cyst "germinating" at both ends in the normal fashion. D 4.

FIG. 14. Rods from rising rod mass, stained with Delafield's hæmatoxylin. $\frac{1}{16}$, 12.

FIG. 15. Encysted rods from mature cyst, stained with hæmatoxylin. $\frac{1}{16}$, 12.

Chondromyces erectus Thaxter.

FIG. 16. Cystophores bearing mature cysts showing habit. C 2.

FIG. 17. Young cystophores on which the cysts are just beginning to form. C 2.

FIG. 18. Mature cyst isolated. D 4.

FIG. 19. Group of rods. $\frac{1}{16}$, 4.

Chondromyces gracilipes Thaxter.

FIG. 20. Mature cystophores and cysts. C 2.

FIGS. 22–23. Mature cystophore and cyst. D 4.

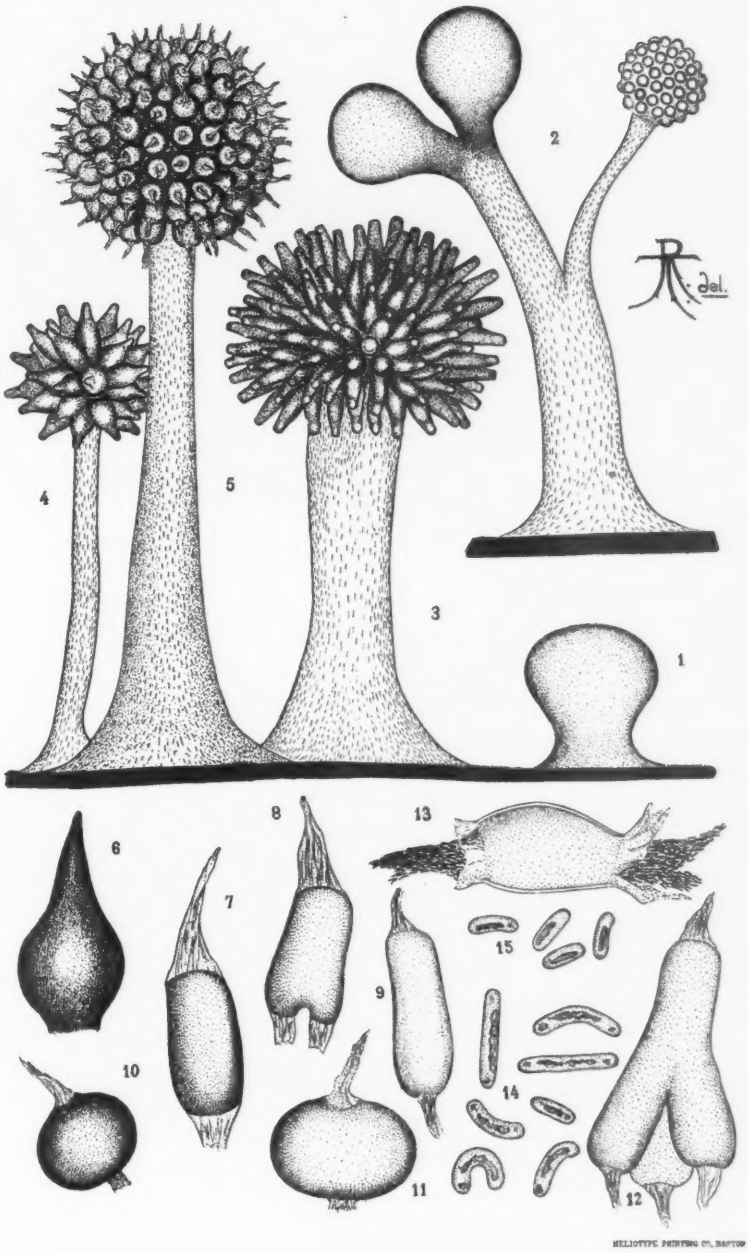
FIG. 24. Group of vegetative rods. $\frac{1}{16}$, 4.

Myxococcus cirrhosus Thaxter.

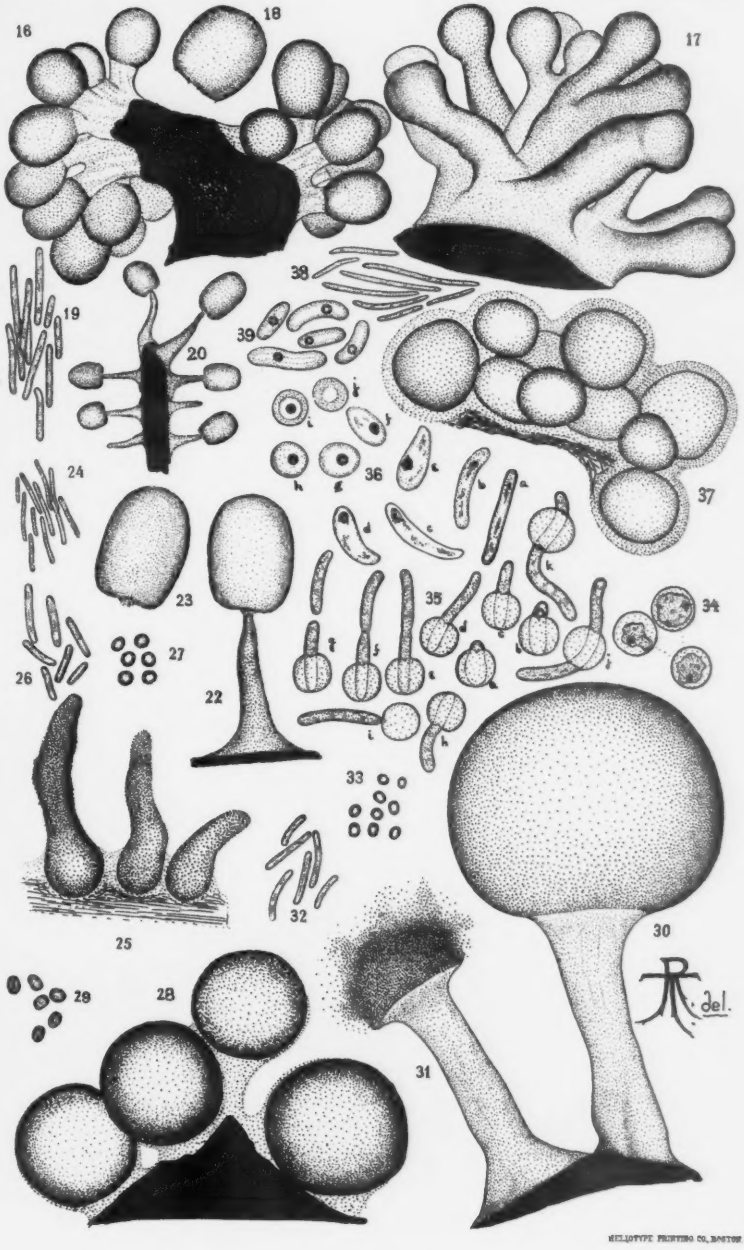
FIG. 25. Three mature spore masses. C 4.

FIG. 26. Group of rods. $\frac{1}{16}$, 4.

FIG. 27. Group of spores. $\frac{1}{16}$, 4.



THAXTER on MYXOBACTERIACEÆ.



THAXTER on MYXOBACTERIACEÆ.

Myxococcus cruentus Thaxter.

FIG. 28. Group of cysts, that at the left showing thickness of cyst wall. C 2.

FIG. 29. Group of spores. $\frac{1}{16}$, 4.

Myxococcus stipitatus Thaxter.

FIG. 30. Stalk with spore mass still intact. C 2.

FIG. 31. Stalk from which the deliquescent spore mass has been removed. C 2.

FIG. 32. Rods. $\frac{1}{16}$, 4.

FIG. 33. Spores. $\frac{1}{16}$, 4.

Myxococcus rubescens Thaxter.

FIG. 34. Three spores preparing to germinate, stained with eosin, from Van Tieghem cell-culture. $\frac{1}{16}$, 12.

FIG. 35. Different stages in the spore germination; *c–g*, division of rod while still adherent within spore wall; *h–i*, rod escaping from spore wall; *j–k*, rod emerging from spore wall on both sides. Drawn from living material in Van Tieghem-cell. $\frac{1}{16}$, 12.

FIG. 36. Successive stages in spore formation; from preparation stained with Delafield's hæmatoxylin, showing deeply staining nuclear-like body and gradual transformation of rod to spherical spore. $\frac{1}{16}$, 12.

Cystobacter fuscus Schroeter.

FIG. 37. Groups of mature cysts removed from substratum. D 2.

FIG. 38. Vegetative rods. $\frac{1}{16}$, 4.

FIG. 39. Rods separated from mature cysts by crushing, and stained with eosin, a nucleus-like body distinct in each.

CONTRIBUTION TO THE LIFE HISTORY OF LILIUM PHILADELPHICUM.[1]

INTRODUCTION.

JOHN M. COULTER.

A group of research students, in connection with a general study of monocotyledons, selected *Lilium Philadelphicum* as a suitable type for somewhat special study. The end in view was to examine those structures so fully described by Guignard for *L. Martagon*, and treated in a supplementary way by subsequent investigators of the same plant. Abundant material of the local *L. Philadelphicum* was obtained, and the cultivated *L. tigrinum* was used also for comparison. The numerous preparations of thirteen investigators gave unusual opportunity for a broad range of observation, so that the facts herein set forth may be regarded as fairly established. As to questions of interpretation, there may well be diversity of opinion, as the present necessities of the case make almost every step in interpretation an inference. It is evident that the association of phenomena will suggest a causal relation, whose reality is plainly only an inference. Moreover, the comparatively obscure structures concerned in cell activity are peculiarly open to misinterpretation, both as to origin and function. The subject, therefore, is one in which dogmatism is singularly inappropriate, and in which every proposed causal sequence of events must be regarded as a suggestion rather than as an established fact.

Inasmuch as this work upon Lilium was but supplementary to the more formal investigation in which each investigator is engaged, my original purpose was to organize under a single caption all of the results that seemed worthy. As the work developed, however, certain parts of it seemed to demand more

[1] Contributions from the Hull Botanical Laboratory. V.

special attention. These special investigations were undertaken by Mr. Chamberlain and Mr. Schaffner, who have made an independent presentation of their results, for which they are entirely responsible. This contribution, therefore, is made up of three distinct and independent papers, each with its own plates, but naturally brought together by the nature of the subject.

My own part is the organization of observations made by the group of students referred to, in so far as they pertain to the embryo sac, fertilization, and the embryo. Mr. Chamberlain, from his own observations, deals with the pollen grain; while Mr. Schaffner presents his own observations and conclusions in reference to certain cytological phenomena connected with the "reduction division" in the embryo sac.

The material used was fixed in Flemming's weaker solution, Merkel's fluid, 1 per cent. chromic acid, 1 per cent. chromic acid with a trace of acetic acid, and picric acid.

Xylol was used almost exclusively to precede the paraffine bath. Serial sections were cut with a Thoma microtome, usually 5 or 10 μ thick, and occasionally but 1 μ thick.

A large number of stains and combinations was used. Cyanin and erythrosin proved excellent for most stages in the development of the macrospore; Delafield's haematoxylin is to be recommended for embryos; safranin with gentian violet and orange G gave good results in staining pollen grains; Heidenhain's iron alum haematoxylin used alone or with erythrosin or orange G gave by far the best preparations for cytological study.

I

THE EMBRYO SAC AND ASSOCIATED STRUCTURES.

JOHN M. COULTER.

(WITH PLATES XXXII–XXXIV)

The results here recorded traverse ground which has become very familiar. It will not be necessary, therefore, to make extended mention of all the phenomena, but to discuss only

certain points which seem to merit comment. It seems best, however, to preserve the sequence of events for the benefit of those who may not have access to the more extensive papers. The students whose observations have supplied the data for this portion of the contribution, and whose individual contributions may be recognized by the initials appended to the different figures, are Otis W. Caldwell, John G. Coulter, Henry C. Cowles, T. C. Frye, Nina D. Holton, Florence M. Lyons, William D. Merrell, Mabel C. Merriman, and Wilson R. Smith.

DEVELOPMENT OF THE EMBRYO SAC.

A single large hypodermal archesporial cell very early makes its appearance, distinguished by its size, contents, and very prominent nucleus (*fig. 1*). No evidence of the cutting off of a tapetal cell, or a division into potential macrospores was detected. The sequence of cell divisions usual in angiosperms is entirely suppressed, and the archesporial cell develops directly into the macrospore (embryo sac). It will be remembered that there are three possibilities in what may arise from the archesporial cell of angiosperms. It may, and apparently usually does, give rise at its first division to a primary tapetal cell and a primary sporogenous cell, each of which may give rise to a more or less extensive cell progeny; or it may, less frequently, give rise to no tapetal region, but play the part of a primary sporogenous cell and divide into potential macrospores; or it may, apparently exceptionally, develop directly into the fertile macrospore. This extreme shortening of the history of the embryo sac, recorded as yet only for Lilium and certain allied liliaceous genera, obliterates the distinctions between archesporial cell, primary sporogenous cell or mother cell, and macrospore, so far as distinct cell existence is concerned, but what the ellipsis involves in nuclear and cytoplasmic changes is worthy of research. Certain it is, that this remarkable cell has a relatively long existence in the uninucleate condition, brought to a close by its rapid enlargement. As there is no tapetum, and no periclinal divisions occur in the epidermis to increase the mass of the nucellus toward the

micropyle, the encroachment of the enlarging macrospore is at first chiefly upon the tissues beneath.

This enlargement is the first step in the "germination of the macrospore," and when the sac has become considerably elongated the first nuclear division occurs near the center, the axis of the spindle being longitudinal (*fig. 2*), and the daughter nuclei passing to their polar positions. A more detailed view of the antipodal end of this first spindle, at an earlier stage, while the chromosomes are still distinct, is given in *fig. 3*, showing the usual transient cytoplasmic radiations about the chromosome group, and the definite relation of the larger ones to the micronucleoli. The cytological phenomena connected with this division, known as the "reduction division," form the subject of Mr. Schaffner's paper. With such an abbreviated history as that of the macrospore of Lilium the division representing the reduction division is evident, but in most angiosperms the place of this special division in the history of the macrospore is not so clear.

Immediately after the placing of the two nuclei the second divisions occur (*figs. 4-8*), the micropylar spindle being transverse, the antipodal one longitudinal. In *figs. 4* and *5* the reduction number of chromosomes is apparent in the micropylar spindle, while a largely increased chromatin mass is apparent in the antipodal spindle. Very soon the resulting nuclei shift their positions more or less (*figs. 7, 7a, 8*), so that the directions of the two spindles are lost. The persistence of the spindle fibers (*figs. 7, 7a*) is a common phenomenon in the embryo sac divisions, and often helps to indicate the shifting of the freed nuclei.

In this second division certain phenomena were noted by Miss Merriman which deserve mention. The occasional occurrence of multipolar spindles in Lilium is well known, and *figs. 9-12* may be taken to represent them. As these spindles are associated with exceptional conditions of the chromatin band, and occurred in a single ovary, they suggest a very unusual and possibly a pathological condition. With the claims made for the relation between the multipolar spindle and the bipolar

spindle, it is interesting to note that among the hundreds of embryo sac spindles of Lilium that passed under our observation, multipolar spindles were found in but a single ovary. In *fig. 9*, representing an antipodal spindle, the chromatin band seems to be arranged in continuous loops the full length of the spindle. In *fig. 10*, representing the micropylar spindle of the same sac, two segments of the chromatin band are arranged also in continuous loops. *Fig. 11*, from another sac of the same ovary, represents a strongly multipolar antipodal spindle, but with the chromatin band broken up into chromosomes; while *fig. 12*, the micropylar spindle from the same sac, shows a continuous looping of the chromatin band. The significance of these phenomena seems quite obscure, and their normal or abnormal character in the case of Lilium can only be ascertained by further investigation. If they represent a normal phase in the development of the bipolar spindle, their rarity would indicate either that it is a peculiarly ephemeral phase, or that it is not easily recognized. If they represent another method of spindle formation their exceptional occurrence might be easily accounted for; and the same may be said of the hypothesis that they represent spindles disorganized by sectioning or reagents. In these same figures (*figs. 9–12*) it will be noticed that the reagents used have brought out abundant striations in the cytoplasm, whose normal or abnormal character may be in question.

Various phases in the eight-nucleated stage of the embryo sac are represented by *figs. 13–16*. The varying directions of the spindles are evident, but in general the synergid spindle is transverse, and the spindles which give rise to the polar nuclei are longitudinal. It is plain that the synergids are sister nuclei, as are also the oosphere and the micropylar polar nucleus. It is also evident from the figures that if direct division occurs among the antipodal nuclei of Lilium our preparations give no evidence of it. An examination of Miss Sargent's figures,[2] which are cited as representing cases of direct division in the

[2] Annals of Botany, 10: 445–447. 1896.

antipodal region of *L. Martagon*, shows that they might be taken for cases of mitosis.

In connection with the spindles of the embryo sac attention should be called to the fact that the spindle fibers thicken in the equatorial plane as if preparatory to the formation of a cell wall (*figs. 14, 15*). This phenomenon has been taken to be another evidence of the descent of this free-celled gametophyte from one of compact tissue.

The condition of things represented by *fig. 17* is difficult to interpret. While it is no unusual thing for a partition wall to be formed at the antipodal end of the sac, a wall at the micropylar end seems worthy of comment. If the nuclear division represented as just taking place is the first division of the definitive nucleus, which seems probable, the other nuclei are easily referred, and it would follow that the synergids are cut off from the oosphere (or oospore?) by a wall.

In our preparations, the fusion of the polar nuclei is so commonly associated with the fusion of the sex cells (*fig. 19*) that the so-called "eight-celled" stage of the sac may be regarded as its ordinary ante-fertilization preparation. *Figs. 18* and *19* represent the fusion of polar nuclei, in the latter case but a small portion of the upper nucleus being shown.

PHENOMENA OF FERTILIZATION.

The pollen tube, as usual, passes between a synergid and the wall of the sac, and then bends more or less sharply towards the oosphere. Its enlarged caliber and more deeply staining contents are associated with the disorganization of the synergid with which it is in contact. If the pollen tube has been directed to the micropyle under the influence of chemotaxis, and the active principle of chemotaxis is a secretion from the synergids, it is interesting to observe that when the tube has reached and passed the synergids it is under the control of an influence powerful enough to bend it sharply towards the oosphere. If the hypothesis of chemotaxis and the origin of the attractive substance are true in this case, it would seem that it does not effect

the essential contact, but brings the tube within the influence of another attraction which immediately directs it to the oosphere. The discharge of a male cell seems to be attended by disorganization and rupture of the tip of the tube (*figs. 19, 20, 24*), as observed by Schaffner in *Sagittaria variabilis*.[3] In *fig. 19* the second undischarged male cell may be seen in the end of the tube in a disintegrating condition; and *fig. 24* represents a case of a remarkably persistent tube and an undischarged male nucleus, the latter being distinctly nucleolated, as late as the second division of the embryo. The synergid not disorganized by the pollen tube persists for some time, as is usual, its nucleus being shown in *figs. 20, 22, 23*.

During fusion the sex nuclei hold no definite position in reference to each other, as is evident from *figs. 19–22*, where certain details concerning nucleoli and chromatin bands may also be noted. It is evident, thererefore, that the position of the fusing nuclei holds no relation to the plane of the first division of the oospore.

In *figs. 20* and *21* the structures figured by Guignard as centrospheres are represented. In these special cases no other structures in the cell bore any resemblance to them, but they were not seen except occasionally in connection with the nuclei in an advanced state of fusion. As no effort was made to demonstrate them, however, this testimony has no special significance. Their frequent association with nuclear phenomena in the higher plants certainly requires explanation, whether the current homology and function be established or not.

DEVELOPMENT OF THE EMBRYO.

Before division the oospore enlarges, elongates, and is not always axially attached to the sac wall (*fig. 23*). At the same time the nucleus enlarges and establishes itself at the free end of the cell. As a consequence, the first division, which is always transverse, results in a small apical cell and a comparatively large and somewhat vesicular basal cell. This basal cell and the

[3] BOT. GAZ. 23: 256. 1897.

subsequent basal region is worthy of remark, and will be noted later. After the first division there is no regular sequence of cell divisions. The second may occur in the basal cell, either transversely (*fig. 25*) or longitudinally (*fig. 24*). That a longitudinal division of the basal cell commonly occurs at some time is evidenced by the later stages of the embryo (*figs. 27–33*). Cell division continues in any region of the embryo and in every direction (*figs. 26–33*). It is impossible to formulate even a general sequence of events or to make any sharp distinction between suspensor and embryo. The amount of the whole embryonic structure which contributes to the completed embryo is variable, and such a thing as a distinctly defined suspensor which may "contribute" to the formation of the embryo does not exist. It would seem better to regard the so-called suspensor not as an organ distinct from the embryo, but rather as a region of the embryo, more or less extensive even in the same species, set apart to serve a temporary purpose. From this standpoint the question as to what the suspensor "contributes" to the embryo, and what the embryo "contributes" to the suspensor, becomes arbitrary and useless refinement. Like much physiological differentiation this may result in a structure externally distinct or it may not. The function of this region of the embryo seems to be to anchor, to absorb, and to relate the embryo properly to its food supply. Therefore, it displays the widest possible variation in extent and structure. The statement that certain plants have no suspensors may or may not be true, but this fact would seem to have no special morphological significance. It has seemed best to me to regard the suspensor not as a phylogenetic rudiment, but as a specialized structure of the embryo adapted to the peculiar conditions of intraseminal development.

The tendency of the basal region of the embryo to spread widely as an absorbent organ in contact with the wall of the sac is very noticeable (*figs. 26–32*). An extreme case at an early stage is represented by *fig. 29*, but cases of still more extensive lateral extension were observed, very suggestive of certain

reported cases of apogamous polyembryony from the nucellar tissue just above the embryo sac. In *fig. 32*, the most mature embryo represented, a distinct development of tissue in the suspensor region is shown, which appears late in the development of the embryo, and is well marked off from the embryo proper by a narrow neck. This suspensor tissue is erythrophilous as compared with the embryo, showing its closer relation to nutritive supplies. From this late development of tissue in the suspensor region, and its great activity, it would suggest its possible association with supposed cases of polyembryony.

DEVELOPMENT OF ENDOSPERM.

It is becoming well known that the first division of the definitive nucleus holds no direct relation to the fusion of the sexual nuclei. It may precede or follow this fusion, or be coincident with it. So far as observed it occurs after the entrance of the pollen tube into the sac, but at almost any time thereafter, apparently related to no special one of those events which follow and which go to make up the process of fertilization. It seems reasonable to suppose that the inciting event is the entrance of the tube. In the case of Lilium the sexual and polar pairs of nuclei were observed to fuse simultaneously, but when division begins the endosperm nuclei divide more rapidly than do the cells of the embryo. When the embryo is but two or three-celled numerous free endosperm nuclei are scattered throughout the embryo sac (*fig. 35*). Later cell walls begin to form in the endosperm. A very interesting phase in the division of the definitive nucleus is shown in *fig. 34*. The remarkably distinct radiations about the forming daughter nuclei seem to be due to the spindle fibers pulled apart, and to other radiations which are similar to those which appear about the forming daughter nuclei in the divisions of the nuclei of the embryo sac which precede fertilization. Such radiations are difficult to interpret, but their distinctness in this preparation could not be exaggerated.

During the development of the embryo and endosperm the

embryo sac enlarges rapidly except at the antipodal end, which is left as a sort of caecum, often thrust to one side, in which may be seen the disintegrating antipodal nuclei (*fig. 35*). Around the narrowed antipodal end of the sac there is developed a very heavy wall, which in itself would seem to be a sufficient reason for the failure of that region of the sac to enlarge.

EXPLANATION OF PLATES XXXII–XXXIV.

The figures are reduced from drawings to about three-eighths of their original size. The combination of objective and ocular is indicated in each case, the initial letters indicating Zeiss, Leitz, Reichert, and Bausch and Lomb. The four combinations used and their magnification in diameters were R$\frac{1}{7}$ R4, 760; B & L$\frac{1}{12}$ immersion R4, 1200; Z$\frac{1}{12}$ immersion L4; Z$\frac{1}{12}$ immersion Z18, 2250. All the figures are from *Lilium Philadelphicum* unless otherwise indicated.

FIG. 1. Tip of nucellus with the single archesporial cell which develops directly into the macrospore. B & L$\frac{1}{12}$ R4.

FIG. 2. First division of the macrospore nucleus, showing radiations about the daughter nuclei, and thickening of spindle fibers in the equatorial region. Z$\frac{1}{12}$ L4.

FIG. 3. Daughter nucleus of the first division at an earlier stage, showing relation of micronucleoli to radiations. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 4. Spindles of the second nuclear division of the macrospore, showing transverse axis and reduction number of chromosomes of micropylar spindle, and longitudinal axis and increased chromatin mass of the antipodal spindle. Z$\frac{1}{12}$ L4. Iron alum.

FIG. 5. Spindles of the second division. Z$\frac{1}{12}$ L4. Iron alum.

FIG. 6. The four nuclei of the second division completed. Z$\frac{1}{12}$ L4.

FIGS. 7–7*a*. The four nuclei, showing persistence of spindle fibers and shifting of nuclei. B & L$\frac{1}{12}$ R4.

FIG. 8. The four nuclei much shifted. B & L$\frac{1}{12}$ R4.

FIG. 9. An unusual antipodal spindle, showing several poles and continuous looping of chromatin band. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 10. The micropylar spindle of the same sac, showing several poles and two masses of continuous looped chromatin band. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 11. An antipodal spindle with numerous poles. Z$\frac{1}{12}$ Z18. Iron alum erythrosin.

FIG. 12. Micropylar spindle of same sac, showing several poles and a continuous looping of the chromatin band. Z$\frac{1}{12}$ Z18. Iron alum erythrosin.

FIG. 13. Spindles of the third nuclear division, showing transverse synergid spindle, longitudinal polar nuclei spindles, and an antipodal spindle. $Z\frac{1}{12}$ L4.

FIG. 14. Same stage, with radiations more evident, equatorial thickening, and a very evident antipodal spindle. $Z\frac{1}{12}$ L4.

FIG. 15. Same stage further advanced. $Z\frac{1}{12}$ L4.

FIG. 16. Completed eight-celled stage. R$\frac{1}{7}$ R4.

FIG. 17. Embryo sac, showing wall at antipodal and micropylar ends, the latter cutting off the synergids, the former one antipodal cell, and the definitive nucleus dividing. B & $L\frac{1}{12}$ R4.

FIG. 18. *L. tigrinum;* polar nuclei fusing. B & $L\frac{1}{12}$ R4.

FIG. 19. *L. tigrinum;* pollen tube with the second male nucleus disintegrating and the tip of the tube ruptured; fusing sex nuclei, the male uppermost; fusing polar nuclei, but the small part of the upper one showing. B & $L\frac{1}{12}$ R4.

FIG. 20. *L. tigrinum;* pollen tube bent sharply towards oosphere, with disorganized tip, the nucleus near its tip being that of persistent synergid; fusing sex nuclei, the male on the left. B & $L\frac{1}{12}$ R4.

FIG. 21. Fusing sex nuclei, with paired centrosomes. B & $L\frac{1}{12}$ R4.

FIG. 22. *L. tigrinum;* fusion of sex nuclei about completed; persistent synergid nucleus above. B & $L\frac{1}{12}$ R4.

FIG. 23. Oospore with broad basal attachment, and the fusion nucleus in apical position; nucleus of persistent synergid still visible. B & $L\frac{1}{12}$ R4.

FIG. 24. Young embryo, showing first division transverse, second division basal and longitudinal; pollen tube with ruptured tip and a remarkably persistent male nucleus. B & $L\frac{1}{12}$ R4.

FIG. 25. Same, but second division transverse. B & $L\frac{1}{12}$ R4.

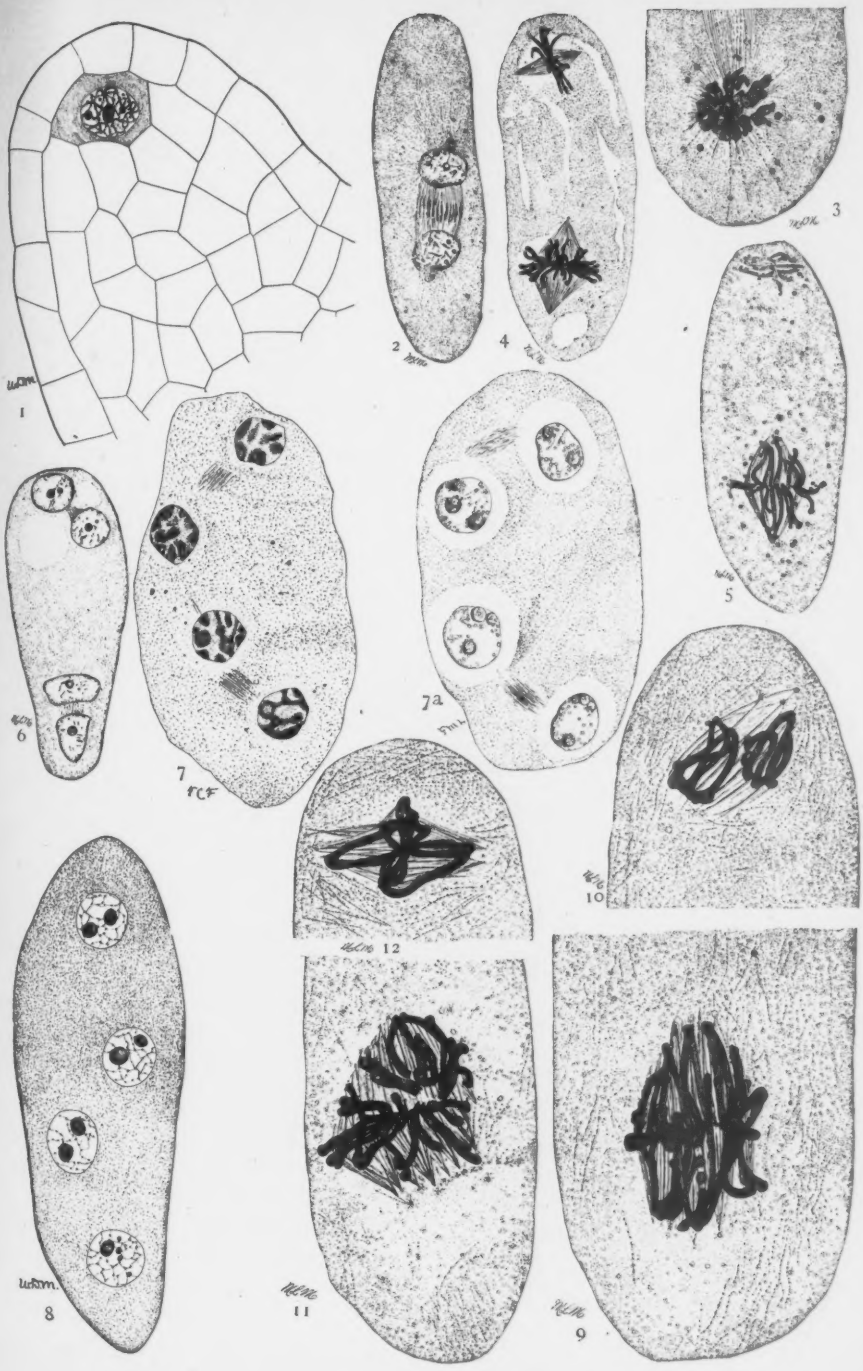
FIGS. 26–30. Young embryos, showing various phases of division and the broadened basal region. B & $L\frac{1}{12}$ R4.

FIGS. 31–32. More advanced embryos. B & $L\frac{1}{12}$ R4.

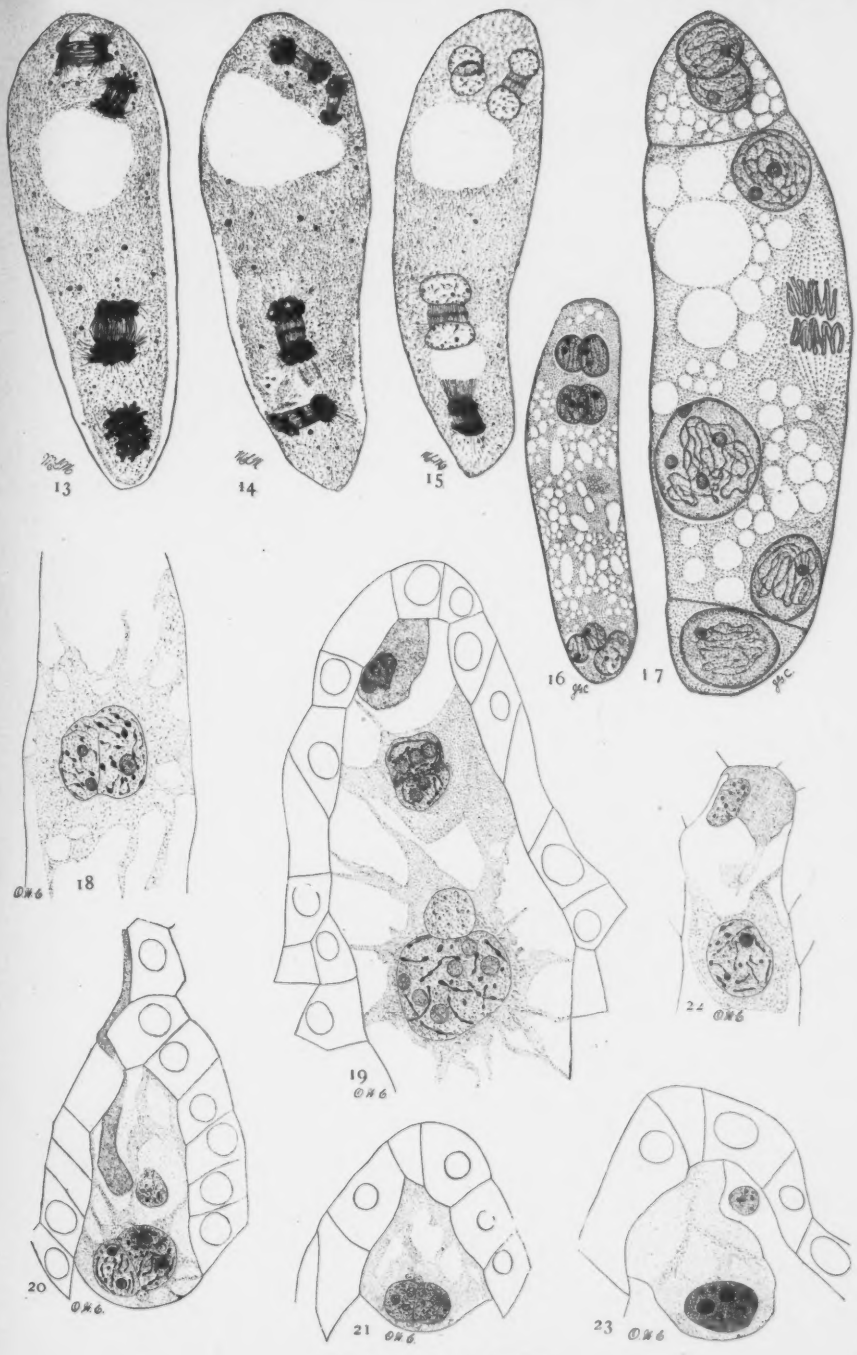
FIG. 33. Advanced embryo, showing development of the suspensor region which is separated by a narrow neck from the embryo proper. R$\frac{1}{7}$ R4.

FIG. 34. *L. tigrinum.* First division of definitive nucleus, showing remarkably prominent radiations about the daughter nuclei. B & $L\frac{1}{12}$ R4. Safranin and gentian violet.

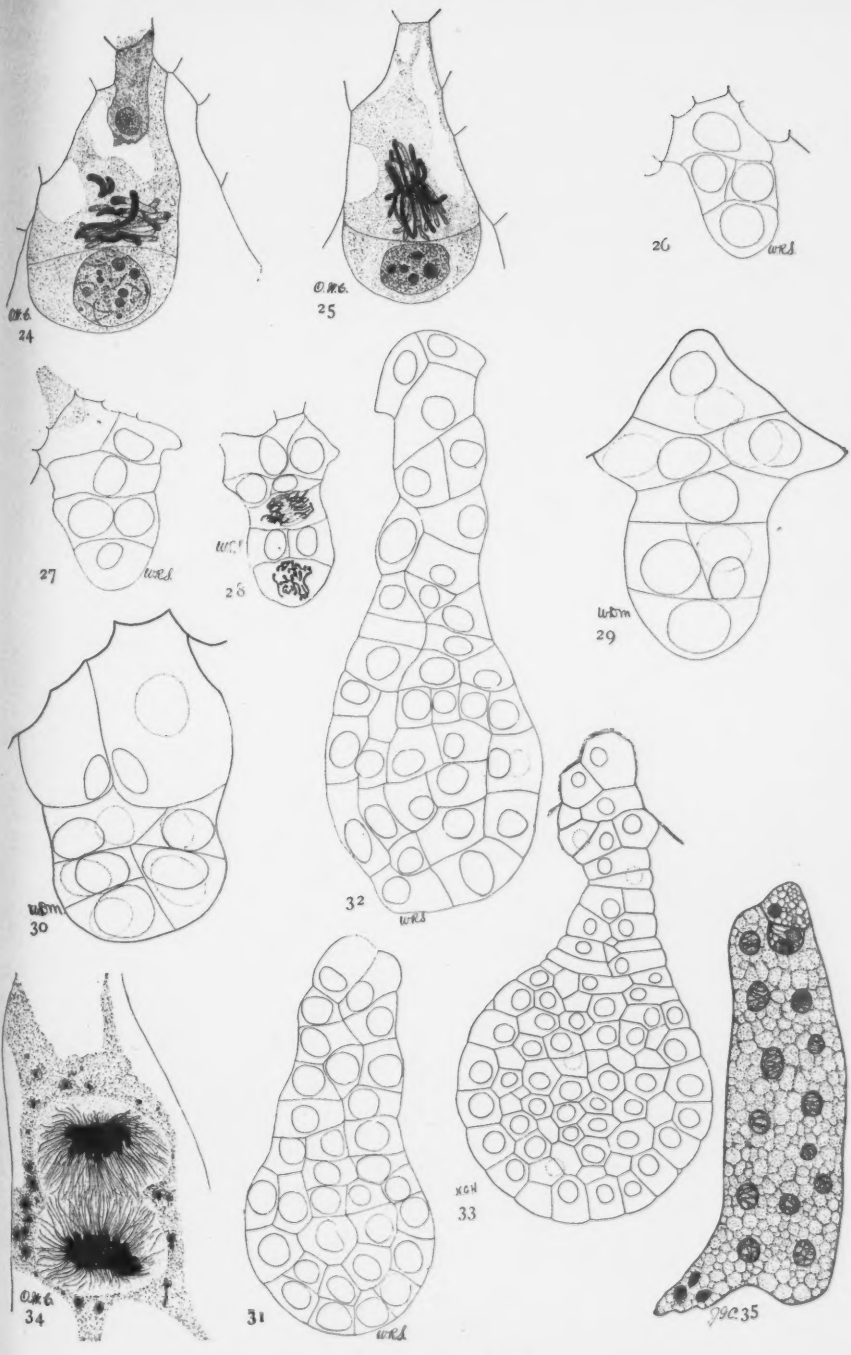
FIG. 35. Embryo sac, showing free endosperm nuclei, the caecum-like antipodal end of the sac containing the three disorganizing antipodal nuclei, and a three-celled embryo. R$\frac{1}{7}$ R4.



COULTER on LILIUM.



COULTER on LILIUM.



COULTER on LILIUM.

II.

THE POLLEN GRAIN.

CHARLES J. CHAMBERLAIN.

(WITH PLATES XXXV–XXXVI)

It is not my purpose to treat this subject in any detail, but merely to note a few of the more essential and critical points. Thanks are due to Professor Coulter for his criticism, and to many advanced students in the laboratory for the privilege of examining several hundred preparations.

HISTORICAL.

During the past thirty years the embryo sac of spermatophytes has received large attention, and its main structures have been figured and described in many species, but the pollen grains, which are of equal importance, have received but scant attention. The most important literature has been furnished by Hartig, Elfving, Dixon, Guignard, Farmer, and Belajeff. Hartig was the first to describe two nuclei in the ripe pollen grain. Strasburger (1884) greatly extended the researches and described the pollen grains of a great variety of species representing the principal groups of angiosperms and gymnosperms. He showed that the smaller of the two cells in the ripe spore is the generative, also that the generative nucleus undergoes division, giving rise to two male nuclei. This division usually takes place in the pollen tube, but in many cases it takes place in the spore, so that the mature spore may contain three nuclei. Elfving saw three male nuclei in the mature spore of *Andropogon campestris*. Strasburger (1884) reports the occurrence of four male nuclei in the pollen tubes of Ornithogalum and Scilla Guignard (1891) says that in *Lilium Martagon* the division of the generative nucleus occurs only in the pollen tube, and that the tube nucleus never divides at all. Strasburger (1884) also makes the general statement that the tube nucleus never divides.

As long ago as 1884 Strasburger discovered that with a

fuchsin-iodine-green mixture the generative nuclei of pollen grains stain green, and the tube nuclei red; but more recently (1892) he has discussed quite thoroughly the staining reactions of the nuclei. The nuclei of the small prothallial cells of gymnosperm microspores are cyanophilous, like generative nuclei. The nuclei of the nucellus surrounding the embryo sac are also cyanophilous. His conclusion is that the cyanophilous condition in both cases is due to poor nutrition of the nuclei, the amount of cytoplasm being small in proportion to the size of the nuclei. On the other hand, the erythrophilous condition of the nuclei of the embryo sac is due to abundant nutrition. As a further proof of the theory it is noted that the nuclei of the adventitious embryos which come from the nucellus of *Funkia ovata* are decidedly erythrophilous, while the nucellus to which they owe their food supply has cyanophilous nuclei.

In division stages nuclei are cyanophilous. From anaphase to resting stage cytoplasm is taken into the nucleus and the cyanophilous condition gradually changes to the erythrophilous, but when a nucleus is prevented from taking nutrition from a large amount of cytoplasm, as is the case with generative nuclei of pollen grains and the nuclei of the small prothallial cells of gymnosperms, the reaction remains cyanophilous. It is an added proof, that in Ephedra the tube nucleus, which has very little cytoplasm about it, is cyanophilous. Strasburger claims that there is no essential difference between the male and female generative nuclei, and observation shows that within the oosphere spermatozoids and other male generative nuclei become erythrophilous, so that the sex nuclei are alike in their reaction to stains. Malfatti and Lilienfeld have proved that these reactions are dependent upon the amount of nucleic acid. Chromosomes during mitosis consist of nearly pure nucleic acid and are intensely cyanophilous; while cytoplasm has little or no nucleic acid and is erythrophilous. There are all gradations between the cyanophilous and erythrophilous conditions, the affinity for basic anilines being in proportion to the amount of nucleic acid.

It is unfortunate that the terms cyanophilous and erythrophilous are becoming established, since the affinity is for basic or acid dyes, and not for blue or red colors. That the terms are misnomers becomes evident when a combination like safranin (basic) and acid green (acid) is used, for the cyanophilous structures take the red, and the erythrophilous the green.

THE MOTHER CELLS AND TETRADS.

Fairly complete series were obtained in *L. tigrinum* and *L. Philadelphicum*, but since the two species showed very similar results only *L. tigrinum* is described.

The mother cell develops its tetrads after the usual manner among monocotyledons. It was my original purpose to make a cytological study of these cells, chiefly with reference to the phenomena involved in the "reduction division," but my attention was diverted to certain structures of the mature spore, which will be hereafter described. However, certain cytological notes obtained may be of interest.

The nuclei of the mother cells in early spirem stages show a single much twisted ribbon with a row of chromatin granules on each edge. In many cases it could be seen that the chromatin granules were arranged in opposite pairs (*figs. 1 and 1a*). These pairs are separated by a longer stretch of ribbon than is figured by Guignard in his description of *L. Martagon*. The granules are usually more or less ellipsoidal in shape, the longer axis coinciding with that of the ribbon. With cyanin and erythrosin the ribbon stains red, and the granules blue. The ribbon splits longitudinally throughout its entire length before it segments into chromosomes (*fig. 1*). The nuclei showed twelve segments of this double thread in all cases in which the number was definitely ascertained. The further history of the chromosomes and the formation of the spindle were not followed.

In the tetrad stage the nuclear thread is not nearly so intricate, and is often spirally wound inside the nucleus, somewhat like a chromatophore of Spirogyra (*fig. 2.*) In many cases it seemed as if even in spirem stages the position of the future

spindle could be predicted. Centrospheres were observed both in the mother cell and tetrad stages.

THE MATURE SPORE.

The microspore usually reaches its full size and the exine acquires its characteristic markings before its nucleus divides. During the growth which precedes this division the nucleus remains approximately in the center of the cell, but just before the division it often moves toward one end of the spore (*fig. 4*). The position of the nuclei, however, often indicates that division has taken place without any such preliminary movement (*fig. 5*). Nearly all the mature microspores of *L. tigrinum* present essentially the conditions represented in *figs. 5* and *6*. The tube nucleus is larger and erythrophilous, while the generative nucleus is cyanophilous. The number and size of nucleoli vary, but as a rule the nucleoli of the tube nucleus are larger than those of the generative nucleus. The chromatin network of the tube nucleus is much finer and more irregular than that of the generative nucleus.

Such cases as *figs. 6–8* are common, and they give the impression that a wall is separating the generative and tube cells. When the generative cell is lenticular and pressed against the wall of the spore it is usually at one end (*fig. 6*), but occasionally it is at one side (*fig. 7*). The cytoplasm of the generative cell was entirely free from starch except in one instance.

Centrospheres were observed in connection with both the tube and generative nuclei. It is comparatively easy to demonstrate centrospheres with the generative nucleus on account of the uniformity in their position and the small amount of cytoplasm in the generative cell. The tube cell is richly supplied with starch, which differs greatly in appearance as different stains are used.

The foregoing applies to most of the pollen grains of the species studied, but occasionally an anther was found which showed very different conditions. The most common variation was the division of the generative nucleus while still within the

pollen grain (*figs. 8, 9*), a condition not uncommon in monocotyledons. More than a hundred such cases were noted in *L. tigrinum*, and about thirty in *L. auratum*, but they are rare in *L. Philadelphicum*. In two cases in *L. auratum* a further division of the generative nucleus was observed, resulting in three male cells (*fig. 10*).

There is abundant negative testimony for the usual statement that the tube nucleus never divides, but hundreds of pollen grains of *L. tigrinum* and *L. auratum* presented such division. In *fig. 12* there are four nuclei, all of which have the characteristics of tube nuclei. One pollen grain was found with eight nuclei, six of which were vegetative and two generative (*fig. 15*). Numerous cases like *figs. 3, 13* and *14* prove that this division is of the direct or amitotic type.

It may be noted in this connection that the cells of the tapetum often contain two, three, or even four nuclei which have been produced by the direct process. No evidence of mitosis was observed in the cells of the tapetum or in connection with the tube nucleus, but it is possible that it occurs in both cases, and that these nuclei may divide by either process. The significance of amitosis seems to be little understood in either animals or plants. The frequency of the phenomenon in pathological tissues has led to the theory that it is due to degeneration. On the other hand, such cases as the internodes of the Characeae suggest that it aids metabolism by increasing the nuclear surface. Its occurrence in gland cells connects it with extreme cell activity. If all cases of amitosis are to have the same explanation it must be much more inclusive than any of these suggested. In Lilium a single tube nucleus seems to suffice in the vast majority of cases. If amitosis is a degenerate condition from mitosis, the division of the tube nucleus might have a phylogenetic significance.

The pollen grains of *L. tigrinum* often showed another variation which seems to be quite important. In *fig. 17*, in addition to the tube and generative nuclei, there is shown a small cell cut off from the end of the spore. A similar condition is seen in

fig. 16, but here the generative cell has effected its ordinary divisions. Over twenty such cases were observed. I have called the cell nuclei marked *g* (*figs. 16, 18*) male nuclei, that is, daughter nuclei from the generative nucleus, because their nucleoli are small, their chromatin network coarse, and in staining they are cyanophilous, that is, they show a preference for basic dyes. Besides, some of these nuclei are surrounded by definite areas of cytoplasm devoid of starch, indicating the organization of the male cell. The tube nucleus, or the several nuclei to which it may give rise, has larger nucleoli, finer chromatin network, and is uniformly erythrophilous. Hence it seems safe to conclude that the nuclei marked *g* in *figs. 16, 18* are generative in origin, and not vegetative like the tube nucleus and its derivatives. The small cell marked *pr* (*figs. 16, 18*) is hard to interpret. Its nucleus is cyanophilous, and its cytoplasm is free from starch. In these respects it resembles the generative nucleus and its derivatives, and if the tube nucleus were the only other nucleus in the spore I should call the small cell a much reduced generative cell. However, a study of all the cases discovered leads me to suggest that the small cell is a prothallial cell, homologous with the single prothallial cell of heterosporous pteridophytes. The small cell cut off from the microspore of *Populus monilifera*, figured but not described in my paper on Salix, adds probability to this hypothesis. If this interpretation is correct, it supports the view that the whole spore development, as it ordinarily appears, is an antheridium. In this case the tube nucleus and its cytoplasm is probably the homologue of the wall cells of such an antheridium as that of Isoetes; the pollen tube would become an outgrowth from the antheridium wall; and the two male cells would homologize with the spermatozoid mother cells. At least it seems out of the question to speak of the pollen tube as the male gametophyte.

Another peculiar phenomenon was noted in *L. tigrinum*. In about twenty cases there was a distinct wall dividing the microspore into two nearly equal parts (*figs. 19-20*). Both cells contained starch, and when each cell contained but one nucleus they

stained alike. In *fig. 20* one of the cells contains two nuclei which seem to represent generative and tube nuclei, and two other such cases were observed. In these cases, also, I am inclined to regard one of the cells as prothallial, and the other antheridial.

EXPLANATION OF PLATES XXXV–XXXVI.

All figures, except *fig. 1 a*, were drawn with an Abbe camera lucida, $\frac{1}{12}$ Bausch and Lomb immersion, and Zeiss ocular 4. The combination gives a magnification of 1010 diameters. *Fig. 1 a* was drawn with $\frac{1}{12}$ Bausch and Lomb, ocular 18 Zeiss. The drawings are reduced one-half by photography: *g*, generative nucleus, *pr*, prothallial cell. *Figs. 3, 9, 10* are from *L. auratum*, all others from *L. tigrinum*.

FIG. 1. Mother cell of pollen grain; the ribbon splitting longitudinally.

FIG. 1 *a*. Small portion of ribbon showing usual shape and position of chromatin granules. Cyanin and erythrosin.

FIG. 2. Young pollen grain from tetrad, showing centrosomes and spiral arrangement of ribbon.

FIG. 3. Mature pollen grain with two tube nuclei and two generative nuclei; one of the tube nuclei suggests direct division.

FIG. 4. Division of primary nucleus of the pollen grain.

FIG. 5. Generative nucleus accompanied by centrosomes; starch quite conspicuous.

FIG. 6. Very common position of generative cell.

FIG. 7. Less common position of generative cell.

FIG. 8. Generative nucleus divided.

FIG. 9. Generative nuclei divided; one generative nucleus and the tube nucleus accompanied by centrosomes.

FIG. 10. Three generative nuclei.

FIG. 11. Tube nucleus divided.

FIG. 12. Four nuclei, all with characters of tube nuclei.

FIG. 13. Three nuclei, two of which indicate direct division.

FIG. 14. Two tube nuclei and one generative nucleus; one of the tube nuclei shows direct division.

FIG. 15. Six tube nuclei and two generative nuclei.

FIG. 16. One tube nucleus, two generative nuclei, and a prothallial cell.

FIGS. 17–18. One tube nucleus, one generative nucleus, and a prothallial cell.

FIG. 19. A definite wall separating the spore into two approximately equal parts.

FIG. 20. Same as preceding, but one part showing what may be interpreted as a generative nucleus and a tube nucleus.

III.

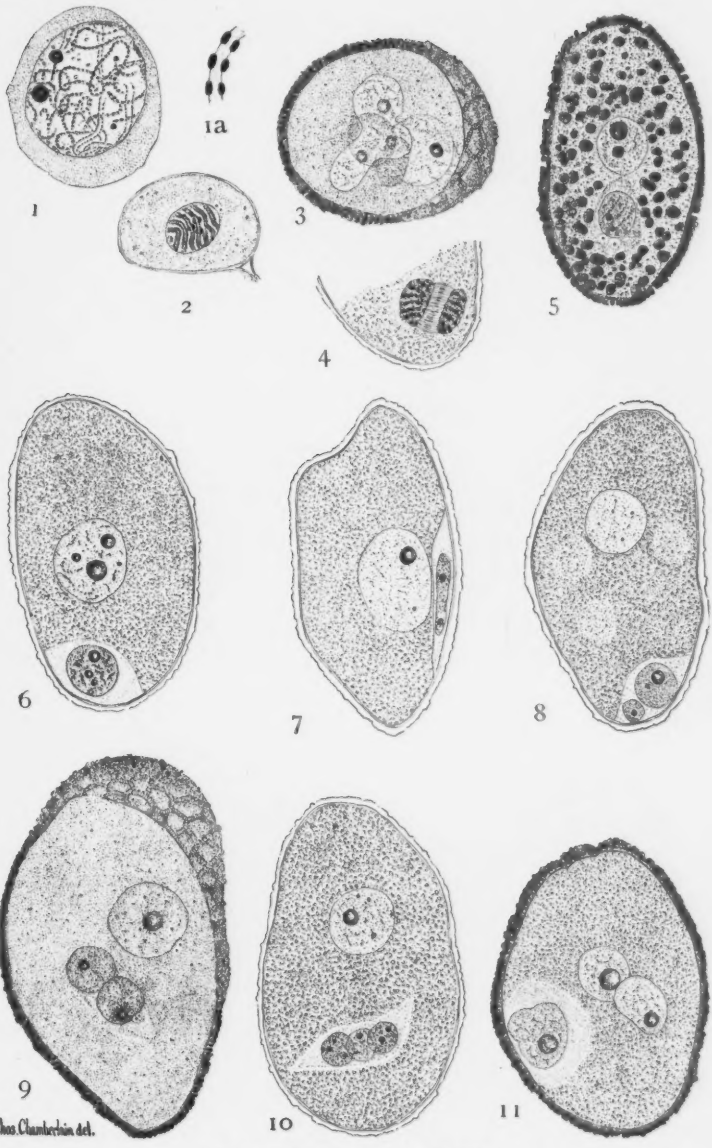
THE DIVISION OF THE MACROSPORE NUCLEUS.

JOHN H. SCHAFFNER.

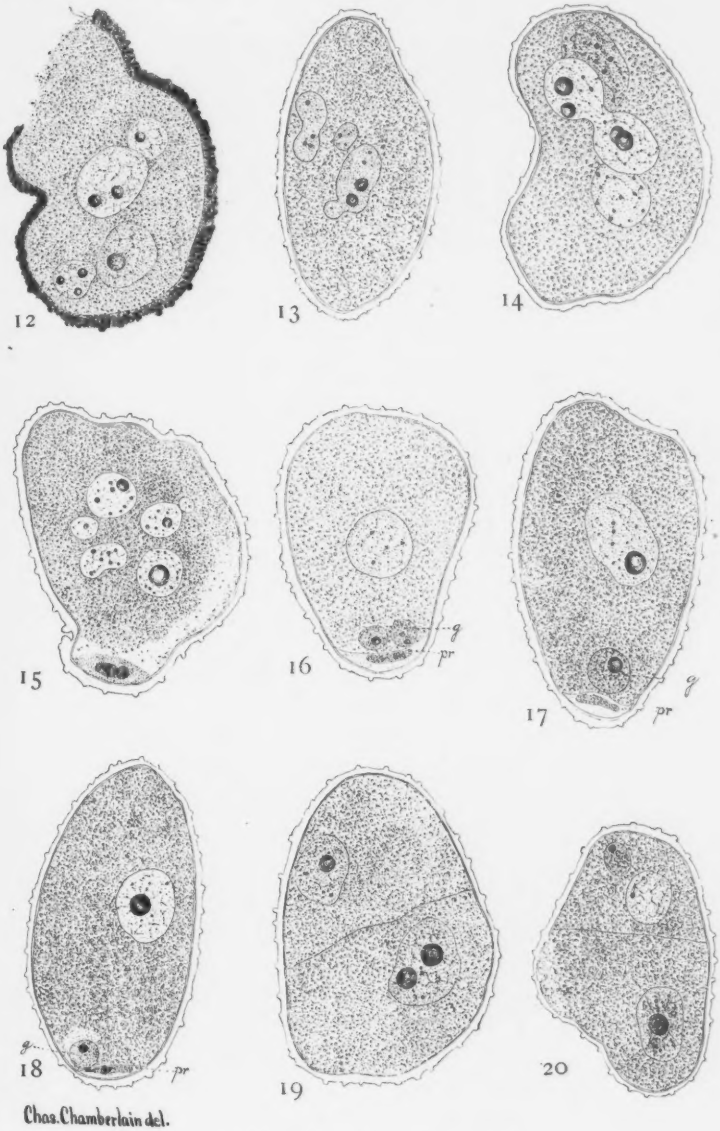
(WITH PLATES XXXVII–XXXIX)

Although a knowledge of the changes which take place in the reduction nuclei of plants and animals is of the utmost importance, and will no doubt aid more than anything else in bringing about a correct interpretation of the facts of heredity, comparatively little has been done in this field, and the observations that have been reported disagree widely. This may be accounted for because of the extreme difficulty of properly preparing suitable material for study, and of correct observation and interpretation of the minute structures concerned. The following work was undertaken because especially favorable material was at hand, and some peculiar variations from what has been received as the normal process of reduction were observed. During the course of the investigation the writer was compelled several times to abandon preconceived notions obtained from the literature of the subject. Whatever, therefore, is presented in regard to the formation of chromosomes and the activities of the nucleoli during karyokinesis has not been the outcome of an attempt to establish evidence which would be agreeable to some hypothesis, but the whole investigation presented an array of facts conclusive to the writer's mind.

My thanks are due to Dr. John M. Coulter for his interest and supervision, as well as to a considerable number of coworkers in the laboratory who kindly permitted me to study and compare their preparations with my own.



CHAMBERLAIN on LILIUM.



CHAMBERLAIN on LILIUM.

ACCOUNT OF INVESTIGATION.

In the young nucellus of *L. Philadelphicum* the archesporial cell soon shows its nature by a difference in size and staining reaction. As is well known, this cell in Lilium develops directly into the fertile macrospore, without cutting off a tapetal cell or dividing further into a number of macrospores. This gives an especially long period of growth for the development of the reduction nucleus. After the macrospore has attained some size its nucleus shows several large nucleoli, usually three in number, with the chromatin rather uniformly distributed and in close connection with the nucleoli. Whether the threads of the network really anastomose or not it would be difficult to determine, but such is the appearance. The threads contain numerous single chromatin granules which are arranged quite regularly, but are not all of the same size (*figs. 1, 1a*).

The chromatin network soon begins to thicken, and the granules grow larger, giving the nucleus a coarser appearance than in the earlier stages. The nucleoli at this stage usually have a homogeneous outer layer, while in the center is a large granular vacuole (*figs. 2, 2a, 3*). At the time when the integuments are just beginning to appear as minute projections on the side of the nucellus, the linin thread of the chromatin network becomes very thick and broad, and the chromatin granules undergo transverse divisions, making the whole network with double rows of chromatin granules instead of the former single row (*figs. 4, 4a*). At this stage, also, the whole chromatin band appears definitely to form a single continuous skein or spirem. At the same time, and even before, important changes are going on in the nucleoli. Sometimes these are of enormous size, with a great granular vacuole in the center. The nucleolus shown in *fig. 5* is larger than the average nucleus of the ovary tissue. In one case (*fig. 6*) such a nucleolus was found with a deep dent on one side. Whether or not this was caused by mechanical injury during preparation it is, of course, impossible to tell. The dent in this nucleolus may be of the same nature as the distortions which produce the so called "sickle stage," but here the

nucleolus was not in contact with the nuclear membrane, but was lying free in the nucleus. The "sickle stage," was seen only in poor preparations, and hence I am inclined to regard it as an artificial product. In many nucleoli at the same stage there are a number of smaller vacuoles, instead of a large central one (*fig.* 7). There is no doubt but that the term vacuole is a misnomer, but for lack of a better one this name will be used for the larger clearer areas in the nucleolus.

After the division of the chromatin granules the entire chromatin band or spirem undergoes longitudinal splitting, producing a double linin thread, each thread containing a single row of chromatin granules (*figs.* 8–9*a*). The double number of chromatin bands makes a very characteristic appearance when compared with the earlier stages before splitting. There does not appear to be any substance connecting the two chromatin bands, the longitudinal fission appearing complete.

At this stage there often appear peculiar radiations or tangential filaments in the cytoplasm. These generally stretch from one side of the cell to the other, passing the nucleus as tangents (*fig.* 8). Whether this appearance was an artificial production or not I could not determine, but it is probable that it is a natural condition, as the threads appeared in numerous sections which did not seem to be otherwise disturbed. At this stage the two centrospheres, which were sometimes seen, still lie close together beside the nucleus.

After the splitting of the chromatin band the two resulting bands now begin to twist on one another, the twisted spirem being in marked contrast with the former parallel arrangement (*figs.* 10, 10*a*). In the meantime the nucleus has enlarged considerably. After the two threads have twisted quite closely together the resulting twisted chromatin band arranges itself so as to form twelve loops, the heads of the loops being close to the nuclear membrane. Each loop contains from one to three twists. At first the double nature of the chromatin band is still very evident (*figs.* 11-11*b*), but later the two linin threads are much more intimately associated, almost giving the appearance

of a single ribbon with an irregular double row of chromatin granules (*figs. 11, 13*). At some point in this stage the so-called "synapsis" is said to occur. The chromatin loops now break apart and lie free in the nuclear area, while at the same time the nuclear membrane has almost entirely disappeared. Wherever the chromosomes were counted they were twelve in number. Thus it will be seen that the spirem first undergoes complete longitudinal fission and then breaks up into half the number of loops or chromosomes that are present in the cells of the sporophyte. The important feature in this pseudo-reduction of the number of chromosomes in the nucleus is not so much the fact that the spirem is cut into twelve parts as that it twists into twelve loops which predetermine the twelve divisions and the twelve chromosomes. The chromatin loops or chromosomes are not all of the same size. Indeed, there is often considerable difference in the lengths of the several chromosomes. In this way there may be considerable diversity in the subsequent reduction of the chromatin granules. Each chromosome then represents a double twisted chain of chromatin granules, and this double thread twisted on itself, so as to make one end of the chromosome a closed loop and the other with two limbs more or less free.

In the meantime the nucleolus becomes filled with a large number of small vacuolate bodies. Each of these bodies has a dark outer part with a light refractive center. Small bodies exactly like those within the nucleolus appear in the nucleus, and as soon as the nuclear membrane has disappeared some of these are also seen in the surrounding cytoplasm (*figs. 14, 15*). The formation of these micronucleoli occurs as follows: The nucleolus sends out a papilla-like projection, into which one of the vacuolate bodies enters and is then separated from the nucleolus by abstriction (*figs. 16-20*). The micronucleoli are thus all separated from the mother nucleolus by a process of budding. Just about the time when the individual chromosomes are formed and the nuclear membrane disappears, cytoplasmic radiations appear all around the nucleus. These threads pass out

at right angles from the nuclear surface and extend to the walls of the cell. They appear like ordinary cytoplasmic radiations with numerous microsomes (*fig. 15*). Whether these threads are the same as the longitudinal threads of earlier stages I did not determine. However this may be, there is no indication of such crossing threads at this stage. If they were present they should have appeared as well as the divergent ones. It may be that the function of these radiations is to carry the micronucleoli out into the cytoplasm. The micronucleoli are perfectly differentiated by various stains. With anilin-safranin and gentian-violet they have a brilliant red appearance, which makes them stand out more prominently in the sections than they do in the figures. With cyanin and erythrosin the nucleoli are blue, while the surrounding cytoplasm and nucleus are red. They are also differentiated by other stains. The mother nucleoli continue to become smaller, and sometimes some of the chromosomes are collected around the nucleoli in such a manner as to suggest that the nucleoli have something to do with the growth of the chromosomes (*figs. 22a, 23a*). This, however, is doubtless merely an appearance, since twelve chromosomes have not much opportunity to avoid contact with two nucleoli in so small a space. Usually the greater number of chromosomes in a nucleus do not lie in the proximity of the nucleoli at all (*figs.21-23*). By the time the chromosomes are ready to be arranged at the equator into the mother star the original nucleoli have entirely disappeared, the small daughter nucleoli or micronucleoli being scattered through the surrounding cytoplasm (*figs. 24, 25*). The micronucleoli show a tendency to become placed near the periphery of the cell and away from the nuclear spindle. However, they are often seen quite close to the poles and on the spindle, where with improper staining they might be confounded with the centrospheres. With proper staining, however, there is no possibility of such confusion, for the nucleoli in the cytoplasm have an entirely different structural appearance from the centrospheres, and also show a different staining reaction. There is no doubt in my mind that the micronucleoli

lying on the spindle have often been mistaken for centrosomes, which would explain the instances where many centrospheres have been reported at the ends of the spindle threads; for it is just at this stage that the micronucleoli would have such a position.

The formation of the spindle was not traced. In the mother star stage one centrosome appears very definitely at each pole (*fig. 26*). During metakinesis the centrosome divides into two (*fig. 36 a*). In the daughter skein stage two large centrospheres are sometimes seen at the poles (*fig. 38*). No special effort was made to bring out the centrospheres, and they were not often seen, but wherever they appeared they showed their normal structure and position.

During the formation of the chromosomes from the chromatin band the twisted loops begin to shorten and thicken, giving the appearance of a single twisted linin thread with an irregular double row of chromatin granules. The linin thread also, especially at this stage, stains a very dark purple or black with Delafield's haematoxylin, exactly like the chromatin granules themselves. At this stage also there is a deposit formed around the chromatin loop which gradually becomes thicker as the chromosome reaches maturity (*figs. 21–23 b*). With Delafield's haematoxylin and erythrosin this deposit stains a light pinkish red, while the chromatin band stains a very dark purple. At a later stage, just before the formation of the mother star, the whole chromosome begins to stain deeper, until it finally has a homogeneous appearance when treated with this double stain, and shows no structure whatever, the whole chromosome appearing like a huge mass of chromatin matter (*figs. 24–27*), and it is necessary to employ other stains to differentiate the chromatin band.

When the chromosomes become arranged on the spindle threads in the equatorial plane they are so situated that the end having the two free ends of the chromatin band are attached to the spindle threads, the loop being turned outward and projecting freely beyond the spindle (*figs. 27–28*). There is no

doubt in my mind that this is the case, although it is difficult to determine, but there is a bare possibility that the chromosomes may be turned the other way. However, their position in the nuclear area, and their appearance and behavior on the spindle, indicate that the loops are turned outward. At this stage also the chromosome is generally stained so dark that no trace of the chromatin band is discernible, but in sections stained with anilin safranin, and gentian violet the central part stains darker and clearly indicates the position of the chromatin band (*fig. 30*). The splitting of the chromosome is gradual (*figs. 29–32*), and consists in the separation or untwisting of the chromatin coil, which is gradually pulled out until it lies like a straight band or rope on the spindle threads (*figs. 33–36*). The untwisting of the chromosome can be seen easily in all stages, and by proper focusing the entire coil can be traced. *Figs. 33–35*, which represent the later phases of this process, do not represent the coiled appearance as well as the sections. After the chromatin coils have straightened, the splitting takes place in the middle of each one at the equator. Thus there is an actual transverse division of the chromosomes, the half of each original chromatin loop passing to opposite poles of the spindle. Each daughter nucleus, therefore, receives about as many chromatin granules as there were in the mother nucleus, and although there is no diminution in the number of chromatin granules, only half of the granules originally present in the mother nucleus are represented in each daughter nucleus. It will be seen that although the chromosomes are not all of the same size and length, yet if the chromatin band breaks at practically the middle point each daughter nucleus receives about the same number of chromatin granules; and since the chromatin granules are the same in number as in the mother nucleus it cannot be proper to speak of a reduction in the amount of chromatin, although only half of the original chromatin granules are represented. There is no reduction in number but a reduction of one-half in kind. Whether there is a reduction in the number of chromatin granules before the egg nucleus is formed must

be determined by studying the subsequent divisions. If there is no such subsequent reduction of granules then it would logically follow that the granules must fuse during the union of the sex nuclei. Otherwise the new sporophyte would contain twice the number of chromatin granules that the old one did.

This splitting of the chromosome first longitudinally and then tranversely, it will be seen, really amounts to the same thing as though the original chromosome were divided into four parts, and corresponds to the so-called "tetrad" stage reported by the zoologists. The word tetrad, however, could not properly be applied here, since a true tetrad does not appear.

After the chromosomes have collected about the poles of the spindle and are beginning to form the daughter skeins, cytoplasmic radiations, similar to those seen around the mother nucleus at the time when the micronucleoli were carried out into the cytoplasm, appear, and the micronucleoli often seem to be attached to them (*fig. 37*). Whether these radiations are organized from the centrosomes at the poles, as might seem possible from *fig. 38*, or are the same as those which surrounded the mother nucleus during the migration of the micronucleoli into the cytoplasm, I could not determine. It might be that they remain constantly in the cytoplasm during metakinesis, and only separated somewhat into two parts. As the daughter nuclei become more complete, the micronucleoli collect around them and begin to enter into the nuclei (*figs. 39, 40*). As they enter the nuclei and fewer are left in the surrounding cytoplasm, the cytoplasmic radiations become less distinct, and they finally disappear altogether when the nucleoli have all entered the daughter nuclei (*figs. 40–43*). The micronucleoli as they enter into the nuclei build up new daughter nucleoli by a continuous process of aggregation and fusion (*figs. 40–43*).

During the divisions of the two daughter nuclei which produce the four-celled embryo sac, the nucleoli act in exactly the same way as has been described for the first division, and the same kind of cytoplasmic radiations arise (*figs. 44–46*). In the divisions which complete the embryo sac, the same process was

observed to occur; so it can be stated without exception that this action of the nucleoli in being thrown out into the cytoplasm and collected again into the daughter nuclei is the normal process for the whole gametophyte generation of *L. Philadelphicum*. Whether this process will be found to occur in the gametophyte generation of all angiosperms, or in all plant cells, is yet to be determined. There is often a marked peripheral placing of the nucleoli in the daughter nuclei which becomes very striking in certain cases where the nuclei lie in just the proper position (*fig. 47*). This would in itself be quite suggestive of the way in which the nucleoli were formed, even if they were not seen to enter from the outside.

The micronucleoli are constantly present in the cytoplasm from the time they leave the nucleus until they enter again. Of course it may be urged that the original micronucleoli are dissolved in the cytoplasm and new ones formed. If this is the case the dissolution of old ones and the formation of new ones must go on simultaneously. It is not intended to contend here that the nucleolus is a permanent cell organ, for more observation is needed for such a generalization. But that the nucleoli pass out and enter again to form new ones in the daughter nuclei cannot be denied. The strongest argument in favor of regarding the nucleolus as a definite body or organ seems to the writer to be the fact that in many plants and tissues the number is constant. Thus in many cases the number in each nucleus is almost absolutely constant. Are such examples of constancy at hand for other excretions or food products? That the number is often variable is no argument against its fundamental character. The number of nuclei in many cells is also exceedingly variable.

During the divisions of the nuclei in the embryo sac the spindle threads undergo a thickening in the middle as though a nuclear plate and cell-wall were to be formed (*figs. 38–41*), and the spindle often persists from one division to another, so that four daughter nuclei may appear to be connected by three spindles (*figs. 45, 46*). This thickening of the spindle threads

would seem to be an inheritance from a true thallus with regular cell walls.

GENERAL DISCUSSION.

Chromosomes.—It has been claimed by botanists, especially Guignard and Strasburger, that during the karyokinetic division of the reduction nucleus in plants the chromosomes undergo longitudinal division just as in ordinary vegetative cells. This has also been maintained by Boveri, Hertwig, and Brauer in regard to Ascaris, where the so-called "tetrad" is said to arise by a double longitudinal splitting of the primary chromatin rod. Recently, however, it has been found by Rückert, Häcker, and vom Rath, that in certain arthropods each "tetrad" arises by one longitudinal and one transverse division of the primary chromosome. This would make a true reduction in Weismann's sense.

Since writing the present investigation the author has read a paper by Calkins[1] in which the formation of "tetrad" chromosomes is described as occurring in the spore mother cells of two ferns, *Pteris tremula* and *Adiantum cuneatum*. The author is quite certain that transverse division occurs in these chromosomes, although he could not tell whether the reduction took place in the first division or in the division following. He thinks, however, that the first division is longitudinal and the second one transverse, so that the reduction would take place in the second division. This, however, is merely an inference, and he seems to have no direct evidence as to when the transverse division takes place, if it occurs at all. Although the work is a very commendable one the author's substitution of zoological for botanical terms seems unwise, since it is still doubtful whether the zoologists have arrived at the exact truth in every case or not. The term "tetrad" in connection with the chromosomes is especially objectionable in botany, since "tetrad" had a definite meaning many years before chromosome "tetrads" were

[1] Chromatin reduction and tetrad-formation in pteridophytes. Bull. Torr. Bot. Club **24**: 101–115. 1897.

thought of. A different term seems advisable in order to avoid the confusion which must arise if it should be introduced into botany.

Mottier[2] has also reported a transverse division of the chromosomes in the pollen mother cells of Lilium. He reports that the pseudo-reduction takes place in the first division and the transverse splitting of the chromosomes in the second. However, his evidence is not very conclusive, and his figures are rather indefinite, so that it is not possible to judge whether his conclusions are justifiable or not. In the case of the reduction nucleus of the embryo sac of *L. Philadelphicum* the divisions which form the macrospores are skipped, so I am not able to generalize or predict what would occur in the normal process where a number of macrospores or microspores are formed.

If we accept Dixon's evidence, it seems probable that the reduction takes place in the first division of the pollen mother cells. In the pollen mother cells of *L. longiflorum*, Dixon[3] found that during the first division the chromosomes sometimes formed a loop which he thinks may be derived possibly from a loop in the original chromatin band, and sometimes they are twisted round each other. He says that while they lie in the equator the two parts of the chromosome are in close contact and seem fused together at their inner extremities, and that during metakinesis the two rod-like portions part from one another. He says: "From the process described it appears probable that each chromosome in this karyokinesis represents two of the previous nuclear divisions which have become more or less completely united end to end." "Thus the reduction in number is effected by an end to end fusion of the chromosomes as Strasburger has already suggested." "The next division by which the pollen tetrads are formed takes place probably according to the normal karyokinesis in plant-cells."

It must be borne in mind, however, that there is at present

[2] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen, Jahrb. f. wiss. Bot. **30**: 169–204. 1897.

[3] The nuclei of Lilium longiflorum. Ann. Bot. **9**: 663–665. 1895.

no possible way of telling in such forms as Lilium what becomes of the individual chromosomes which go to make up the spirem of the reduction nucleus. The spirem is a continuous thread before it breaks up, and to say that each piece represents two of the former chromosomes is a mere assumption dangerous to make. It is just as probable, so far as we know, that the breaking may take place anywhere, and that the individuality of the former chromosomes is entirely lost.

Turning now to Guignard's [4] figures of the division of the pollen mother cells and embryo sac nucleus of *L. Martagon*, we find a wonderful agreement in his figures with my own. If the chromosomes represented in Guignard's *figs. 48* and *49* had the chromatin band closed into a loop they would agree exactly with mine from this stage on. His figures indicate that the process of splitting was the same as I have determined, but that he was misled by thinking that his double chromatin band was open at both ends. And were no conflicting evidence at hand I should say that his figures of the divisions in the pollen mother cells establish a transverse splitting of the chromosomes for the first division of the pollen mother cells and not for the second. Whatever the fact may be, it is to be hoped that evidence on this point will soon be offered which will relieve the present uncertainty.

It will be seen, as already stated, that in *L. Philadelphicum*, because of longitudinal splitting of the spirem and its subsequent transverse division during metakinesis, we have to do with exactly similar phenomena as those which have been described for "tetrad" formation, although the chromosome never gives any appearance of a separation into four distinct parts, but as has been described forms a double twisted coil. The early longitudinal division of the spirem, before it breaks up into the reduced number of chromosomes, has also been found by vom Rath [5] in the insect *Gryllotalpa vulgaris*.

[4] Nouvelles Études, etc. Ann. Sci. Nat. Bot. VII. **14**: 163–296. 1891.

[5] Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris. Arch. f. Mik. Anat. **40**: —. 1892.

Miss Sargent[6] has recently studied the division of the reduction nucleus of *L. Martagon*, but was unable to detect any transverse division of the chromosome, although some facts observed by her point that way. She has much to say in regard to synapsis, but I am fully convinced that many of the appearances she describes were due to poor treatment of the material. In fact, I regard the so-called stage of synapsis as simply a product of poor preparation. In none of my better preparations have I found such an appearance, in fact it was so rare in the stages where it is reported to occur that I should have missed it altogether had I not made a careful search for its appearance, although I had a large number of my own preparations and had the privilege of looking over a large number put up by others in the laboratory. In the stage when the contraction usually occurs the chromatin band is quite free within the nuclear membrane, since it is at this time twisting and orienting itself to form the twelve chromatin loops. Everything therefore is favorable for an artificial contraction. When the contraction does take place it is often exactly in the middle of the nuclear area, and sometimes it occurs in such a manner that the large nucleolus is left entirely free in the nuclear area away from the mass of chromatin. Miss Sargent's explanation, therefore, that the chromatin contracts around the nucleolus in order to keep this "washy" looking body from escaping beyond the confines of the chromatin because of its supposed dissolution at this period, I venture to regard as erroneous. In various other plants I have seen contractions of this sort, but always in very poor preparations, from which it would not be wise to draw conclusions, and I believe that I am safe in saying that the nucleolus is much more often free in the nuclear area than caught in the contracted meshes of the spirem.

Although a transverse division of the chromosome, or a true reducing division, is here established for the macrospore of *L. Philadelphicum*, the writer does not wish to be understood as

[6] The formation of the sexual nuclei in Lilium Martagon. I. Oogenesis. Ann. Bot. **10**: 445–477. 1896.

thinking that this will give any support to Weismann's theory of heredity. It has been shown already by certain botanists that this theory breaks down entirely in the case of plants. Whether Strasburger's theory of reduction is correct or not the future must decide. We are evidently not yet ready to establish a theory of reduction, as we are dealing with phenomena concerning which hardly two observers agree.

Nucleoli.—Apparently no structure in the cell is so little known as the nucleolus, and one needs only examine the literature of the subject to discover how little has been done that can stand the test of criticism. One of the most common ideas in regard to this body was that it represents a globule of food material which can be used by the nucleus or cell in general as occasion demands. Strasburger,[7] in 1895, suggested that the substance of the nucleoli serves for the construction of the spindle threads, but it is doubtful whether there ever was any very strong evidence in favor of such a view. Went,[8] in 1887, saw in the endosperm nuclei that the nucleoli were lying in contact with the chromatin threads, and he thought the substance of the nucleoli was taken up by the chromosomes. It is very doubtful, however, whether any part of the nucleoli ever goes to aid in the formation of the chromosomes. Tangl[9] was the first to observe the extrusion of the nucleolus into the cytoplasm. This was seen in the pollen mother cells of *Hemerocallis fulva*. Karsten[10] attempted to show that the centrosomes had their origin in extruded nucleoli. It was soon shown by Guignard and others, however, that this was not the case, and that Karsten did not see true centrosomes at all. Belajeff[11] has the nucleoli passing out into the cytoplasm, but he claims that they are dissolved and that new ones arise in the daughter nuclei.

[7] Karyokinetische Probleme. Jahrb. f. wiss. Bot. **28**: 151–204. 1895.

[8] Beobachtungen über Kern- und Zelltheilung. Ber. d. d. bot. Ges. **5**: 247. 1887.

[9] Die Kern- und Zelltheilung bei der Bildung des Pollens von *Hemerocallis fulva* L.. Denkschr. d. Kais. Akad. d. Wiss. zu Wien. **45**: 65. 1882.

[10] Ueber Beziehungen der Nucleolen zu den Centrosomen bei *Psilotum triquetrum*. Ber. d. d. bot. Ges. **11**: 555–562. 1893.

[11] Zur Kenntniss der Karyokinese bei den Pflanzen. Flora —: 436. 1894.

A. Zimmermann[12] found that at the beginning of nuclear division the nucleoli pass out of the nucleus into the cytoplasm, and that later in the metaphase of the division they are again taken up into the daughter nucleus. He advances the proposition "omnis nucleolus e nucleolo." This process was observed in the pollen mother cells of *Lilium Martagon*, where the nucleoli are said to break up into numerous small granules. Zimmermann also observed the extrusion of the nucleoli into the cytoplasm in *Hyacinthus candicans*, *Fritillaria imperialis*, *Equisetum*, and *Psilotum*. The extrusion was also found in the primary embryo sac nucleus of *Lilium Martagon* and *Fritillaria imperialis*, in the vegetative cells of the root tips of *Vicia faba*, and in the stem tips of *Phaseolus communis* and *Psilotum triquetrum*. Rosen[13] has also found nucleoli in the cytoplasm of the cells of roots of Hyacinthus. Zimmermann's observations were immediately disputed by Guignard, Humphrey, and Strasburger. Humphrey[14] considers that the phenomena recorded by Zimmerman are the results of manipulation.

Zimmermann believes that the nucleoli which are thrust out into the cytoplasm return again into the daughter nuclei. In his last work[15] he still thinks that this occurs in some cases, but that it may not be universal. He considers that there is a fusion of the nuclei even in the cytoplasm, and I have also found that this seems to be the case, although generally no fusion of micronucleoli occurs until they have entered the daughter nucleoli. Whether the nucleoli are real organs of the cell of course still remains an open question, but for the divisions that take place in the embryo sac of *Lilium Philadelphicum*, my own study assures me that in the prophase of nuclear division the nucleoli give rise to a large number of micronucleoli which are carried out into the cytoplasm, and in the metaphase of the

[12] Ueber das Verhalten der Nukleolen während der Karyokinese. Beiträge zur Morphologie und Physiologie der Pflanzenzelle **2**: 1–35. 1893. Tübingen.

[13] Beiträge zur Kenntniss der Pflanzenzellen. Beitr. z. Biol. der Pflanzen (Cohn). **7**: 225–312. 1895. [Heft 2].

[14] Nucleolen und Centrosomen. Ber. d. d. bot. Ges. **12**: 108–117. 1894.

[15] Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena. 1896.

division they are again collected into the daughter nuclei, where by repeated fusions they form the large nucleoli of the mature daughter nuclei.

Cytoplasmic radiations and spindle threads.—The tangential threads which I observed in the early stages of the development of the macrospore nucleus are no doubt the same as those recently described by Mottier and others as being the beginnings of the achromatic spindle. They do not converge to definite points, however, but pass almost in straight lines across the cell from one wall to the other, the greater number appearing to pass in a direction longitudinal to the long axis of the growing macrospore. From their varying direction it follows that there must be numerous points of intersection, but it does not appear that any number intersect at a given point. At a later stage, in very fine sections, these radiations do not appear at all, but a new set of cytoplasmic threads appear diverging in every direction, and at right angles to the nuclear surface. These radiations are the same as those figured so beautifully by Guignard. Whether these diverging radiations represent the same structures as the earlier tangential threads or not I could not determine. They were never seen to converge to definite points, but I can easily imagine how in a contracted condition of the cytoplasm they might give such an appearance. My belief is that the real purpose of these radiations is to carry out the micronucleoli into the cytoplasm. They appear just when the micronucleoli begin to migrate, and it is difficult to imagine how the nucleoli could pass outward unless carried by streams of cytoplasm.

The formation of the spindle was not followed, but in the mother star stage the spindle is always bipolar and ends in a definite point. No appearance of a multipolar spindle was ever observed, unless in cases where the spindle had been cut or torn Even granting that the spindle is formed as Farmer states, there may still be two unseen centrospheres toward which all the small poles of the multipolar spindles are attracted. It is inconceivable to the writer how a definite bipolar spindle should

always be formed from several minor poles which extend in every direction, without the control of some body or other influence at a definite point. The writer has preparations in which very definite centrospheres are to be seen at the poles of the mother star. These preparations have been examined by a large number of experienced observers at more than one laboratory, so that the sweeping assertion that no such bodies have ever been observed in the higher plants is certainly unwarranted.

In this connection it might also be proper to speak of the large spherical bodies figured by Farmer[16] on his multipolar spindles in the pollen mother cells of *Lilium Martagon*. There is no doubt in my mind, from the description and figures, that these were micronucleoli, and had nothing whatsoever to do with the spindle, especially as he speaks of granules which are colored by those stains which differentiate the chromatic elements of the nucleus.

It must be remembered that it is an impossibility to find the spindle converging to a single pole when the nucleus and spindle have been cut into half a dozen slices, and more often cut diagonally than longitudinally. No one denies that multipolar spindles are to be seen in such cases. But it will be remembered that most of these so-called multipolar spindles have been reported in just such cases, nearly always in connection with the enormous nuclei of the pollen mother cell or the reduction nucleus of the embryo sac. The cell and its contents would have to be made of steel or flint if it were to preserve its centrospheres and poles in proper position after having been cut into half a dozen or more sections. It is even claimed that in the thinnest sections it is easily seen that the spindle does not come to a pole, which is certainly not surprising.

The numerous radiations which appear around the daughter nuclei, if the suggestion is correct for those around the mother nucleus, are for the purpose of returning the micronucleoli into the daughter nuclei. As has been said, the micronucleoli are

[16] On nuclear division in the pollen mother cells of *Lilium Martagon*. Ann. Bot. 7: 392–396. 1893.

often seen on these radiations on their return, and the radiations disappear as soon as all the micronucleoli have entered the daughter nuclei, as if there remained no further function for them. There is no spindle to be formed with which they might be associated, as is the case with those around the mother nucleus.

That the central spindle threads are not necessary for the formation of the daughter nuclei is shown by the fact that the entire central spindle may persist in the cytoplasm for several divisions. At some future time the writer hopes to discuss the origin of the spindle threads and their relations to the centrosomes.

SUMMARY.

1. In *Lilium Philadelphicum* the archesporial cell develops directly into the macrospore, and its nucleus during the first-division appears with twelve chromosomes, or half the number which are present in the vegetative nuclei. At quite an early stage in the development of the macrospore the linin thread of the chromatin network begins to thicken, and the chromatin granules undergo transverse fission. After division of the chromatin granules the whole chromatin band undergoes longitudinal splitting, and the double threads thus produced begin to twist upon each other. This twisted band finally manifests itself as a single continuous spirem, which doubles up and twists into twelve loops. The twelve loops break apart and give rise to the twelve chromosomes. The two linin threads with their granules, which compose the loop, continually become more intimately associated, so that the loop appears like a single linin thread with two irregular rows of chromatin granules. The chromatin loops become shorter by contraction, and receive a thick deposit of some substance which stains light at first but later takes the same color as the chromatin, giving the chromosomes the appearance of homogeneous, somewhat irregularly bean shaped bodies, in which the original chromatin loop is rarely visible The chromosomes arrange themselves in

the equatorial plane in such a manner that the end containing the two free ends of the original chromatin loop is in contact with the spindle fibers. The fibers gradually pull the chromosomes apart in such a manner that the original chromatin loop is untwisted and finally cut in two by a transverse division. Thus the parts of each chromosome which pass to the daughter nuclei represent transverse halves of the original chromatin loop, formed from about one twelfth of the double spirem.

2. The nucleus at first usually has about three nucleoli, each with one or more large granular vacuoles. After the longitudinal splitting of the chromatin band there arise in the nuclei numerous small vacuolate bodies. These are successively abstricted from the mother nucleolus by a process of budding, and give rise to numerous micronucleoli, which all pass out into the cytoplasm before the formation of the mother star, and later, at about the beginning of the close daughter skeins, these micronucleoli all pass back into the daughter nuclei, and by aggregation form the new nucleoli of the daughter nuclei. This process is repeated for every division of the female gametophyte.

3. At about the time of the division of the chromatin granules, there appear in the cytoplasm peculiar cytoplasmic threads, which pass from one side of the cell to the other, and are mostly tangent to the nucleus. At a later stage, at about the beginning of the nucleolar migration, these threads have disappeared, and numerous radiating threads pass out at right angles from the nuclear surface and extend to the cell walls. These radiations seem to hold some relation to the migration of the micronucleoli. Similar radiations appear around the daughter nuclei, and the micronucleoli, as they are drawn into the daughter nuclei, seem to be in contact with these cytoplasmic threads.

4. Two centrospheres appear beside the resting nucleus, and in the mother star stage a single centrosphere appears at each pole of the spindle; while a little later, during metakinesis, a centrosphere appears at each point with a double centrosome.

In the daughter skein stage there are two centrospheres at each pole, which are often quite distinct and can easily be differentiated from the micronucleoli.

THE UNIVERSITY OF CHICAGO.

EXPLANATION OF PLATES XXXVII–XXXIX.

All the figures are reduced to three-eighths of their original size. The magnification given with each figure is the original magnification of the drawings before reduction.

FIG. 1. Part of a young nucellus showing the archesporium developing directly into a fertile macrospore. Anilin-safranin, gentian-violet. × 1200.

FIG. 1*a*. A small part of the chromatin network showing a single row of chromatin granules on the linin threads. × 2250.

FIG. 2. Nucleus a little older than in *fig. 1*. The chromatin network seems to be in close connection with the nucleoli. Chromatin granules considerably larger. Anilin-safranin, gentian-violet. × 1200.

FIG. 2*a*. Linin thread with chromatin granules.

FIG. 3. Nucleolus; the usual appearance before the division of the chromatin granules. Delafield's haematoxylin, erythrosin. × 2250.

FIG. 4. Section of nucleus at the stage when the integuments are just beginning to appear. The linin thread is very thick and the chromatin granules are dividing transversely. There were four large nucleoli in this nucleus. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 4*a*. Small piece of the linin thread showing arrangement of the chromatin granules. × 2250.

FIG. 5. An enormous nucleolus, with large central vacuole, which has a granular structure. Just before division of the chromatin granules. Anilin-safranin, gentian-violet. × 2250.

FIG. 6. Nucleolus about the same stage as *fig. 5*, with a depression or dent on one side. Anilin-safranin, gentian-violet. × 2250.

FIG. 7. Nucleolus with several vacuoles; same stage as *figs. 6* and *7*. Anilin-safranin, gentian-violet. × 2250.

FIG. 8. Macrospore with nucleus containing double chromatin bands produced by longitudinal splitting. Two centrospheres on one side of the nucleus. The cytoplasm contains peculiar radiating strands. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 8*a*. Section of the same nucleus showing another nucleolus. × 1200.

FIG. 8*b*. Short piece of double chromatin band. × 2250.

FIG. 8*c*. Double chromatin band a little wider than *fig. 8b*. × 2250.

FIG. 9. Thin section of a macrospore with nucleus containing double rows of chromatin bands. The nucleolus shows a number of small vacuoles. Anilin-safranin, gentian-violet. × 1200.

FIG. 9*a*. A single thread of the double chromatin band showing the arrangement of the chromatin granules on the linin thread. × 2250.

FIG. 10. Nucleus in which the double threads of the chromatin band are beginning to twist on each other. Anilin-safranin. gentian-violet. × 1200.

FIG. 10*a*. A double twisted chromatin band crossed by a single one. The other part was very likely cut away. × 2250.

FIG. 11. Section of a nucleus in which the double twisted chromatin band has twisted into twelve loops with the heads toward the nuclear membrane. Anilin-safranin, gentian-violet. × 1200.

FIG. 11*a*. Adjoining section of the same nucleus. × 1200.

FIG. 11*b*. Two loops from *fig. 11*. × 2250.

FIG. 12. Chromatin loop a little later than *fig. 11*. The two linin threads have twisted more closely together, presenting more nearly the appearance of a single band. Anilin-safranin, gentian-violet. × 2250.

FIG. 13. Thin section of macrospore showing chromosomes immediately after the breaking up of the chromatin band into the twelve chromosomes. The nuclear membrane has almost disappeared. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 14. Section of macrospore showing the double nature of the chromatin loops, a large vacuolate nucleolus, and three micronucleoli, one of which has passed beyond the nuclear limits. Anilin-safranin, gentian-violet. × 1200.

FIG. 15. Section of macrospore showing fine cytoplasmic radiations extending outward from the nucleus. In the nuclear area is one large nucleolus and numerous micronucleoli. Some of the micronucleoli have traveled far out into the cytoplasm. Anilin-safranin, genatin-violet. × 1200.

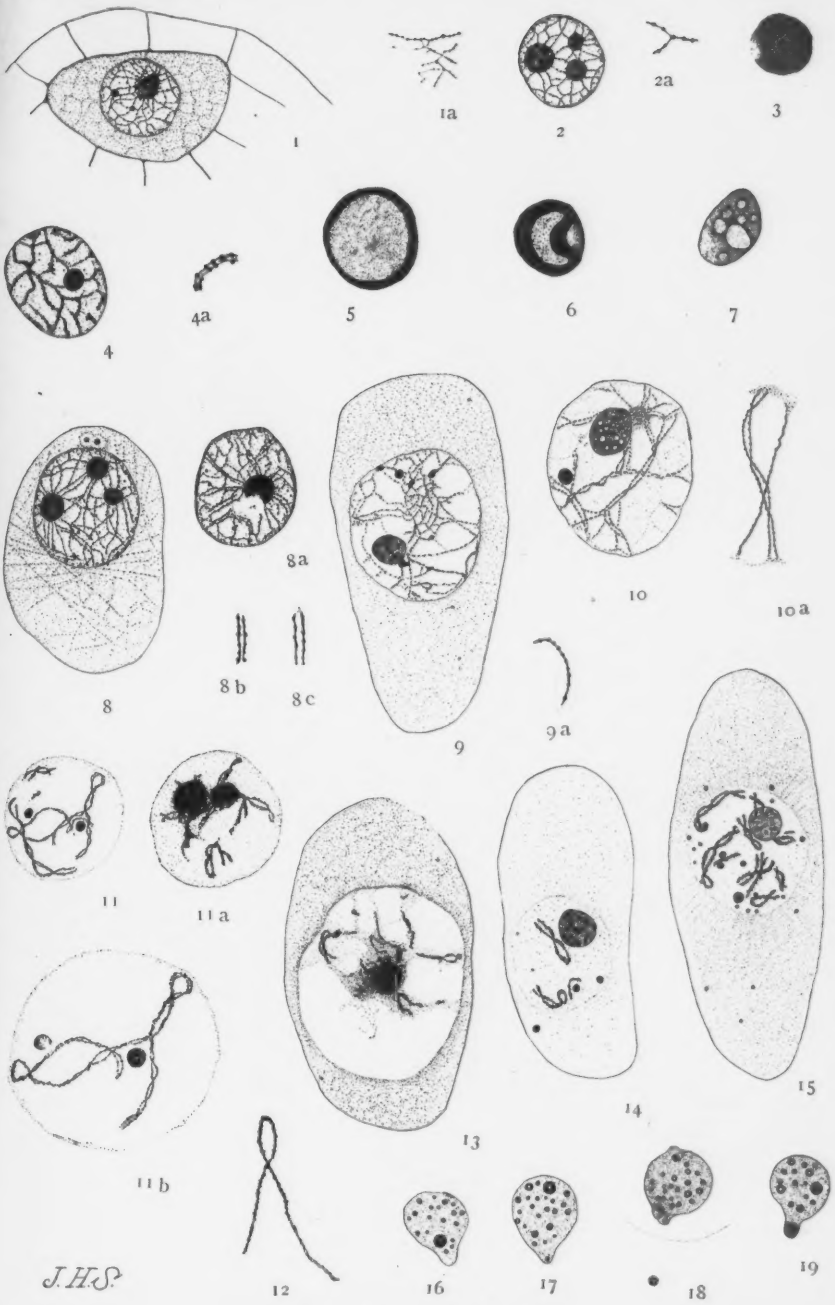
FIG. 16. Nucleolus with vacuolate bodies; just before the breaking up of the chromatin band. Anilin-safranin, gentian-violet. ×2250.

FIG. 17. Nucleolus with vacuolate bodies. Anilin-safranin, gentian-violet. × 2250.

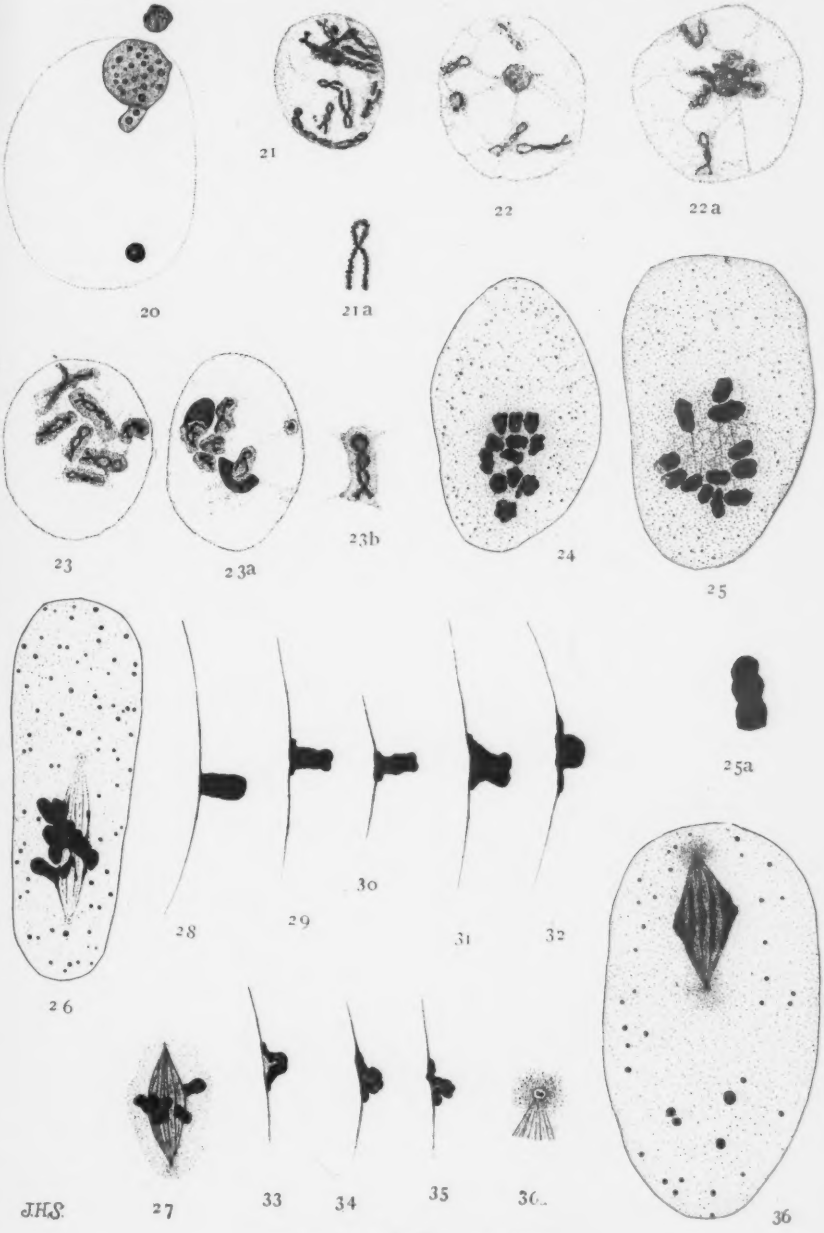
FIG. 18. Nucleolus with small spherical vacuolate bodies. A small nucleolus lying outside of the nuclear boundary. Anilin-safranin, gentian-violet. × 2250.

FIG. 19. Nucleolus with one of the vacuolate bodies apparently being extruded. Anilin-safranin, gentian-violet. ×2250.

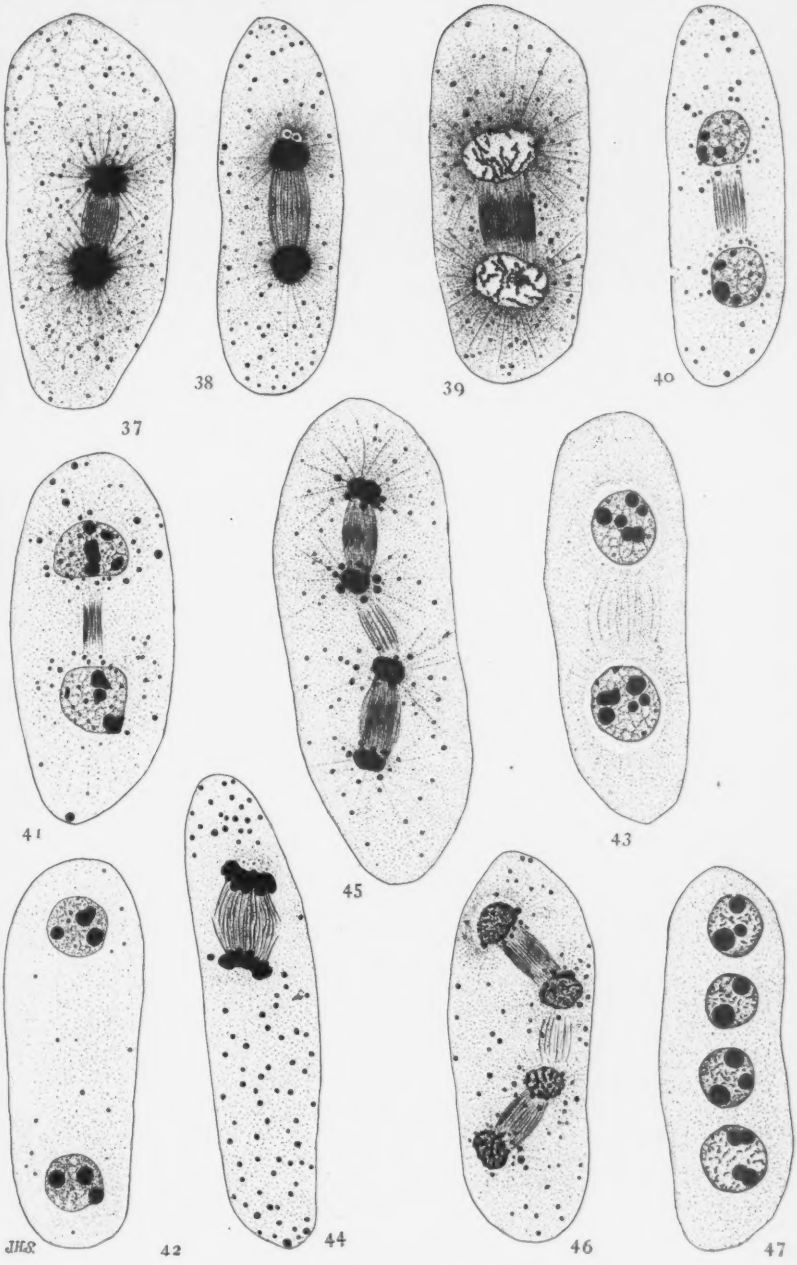
FIG. 20. Outline of a nucleus, showing one large vacuolate nucleolus and a micronucleolus within the nuclear area, a small vacuolate nucleolus outside. Anilin-safranin, gentian-violet. × 2250.



SCHAFFNER on LILIUM.



SCHAFFNER on LILIUM.



SCHAFFNER on LILIUM.

FIG. 21. Nucleus, showing twelve developing chromosomes. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 21*a*. A single chromatin loop showing the double arrangement of the chromatin granules. × 2250.

FIG. 22. Section of nucleus with five chromosomes and a vacuolate nucleolus. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 22*a*. Adjoining section of the same nucleus showing the remaining chromosomes, five of which are clustered around a nucleolus. × 1200.

FIG. 23. Section of a nucleus, showing seven chromosomes. The chromatin loop is covered with a thick layer of material, which is stained a light purplish red, while the chromatin band itself is a very dark purple. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 23*a*. Adjoining section of the same nucleus, showing two nucleoli and five chromosomes. × 1200.

FIG. 23*b*. A single chromosome. × 2250.

FIG. 24. Macrospore, showing twelve chromosomes and numerous micronucleoli in the surrounding cytoplasm. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 25. Macrospore with twelve chromosomes and numerous micronucleoli in the surrounding cytoplasm. A little later stage than *fig. 24*. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 25*a*. A single chromosome stained a very dark purple with a homogeneous appearance throughout. × 2250.

FIG. 26. Macrospore with nucleus in the mother star stage. Centrospheres at the poles of the spindle and numerous micronucleoli in the surrounding cytoplasm. Anilin-safranin, gentian-violet. × 1200.

FIG. 27. Mother star showing the arrangement of the chromosomes on the spindle threads. Iron-alum, haematoxylin. × 1200.

FIG. 28. Chromosome from mother star. Cyanin, erythrosin. × 2250.

FIG. 29. Chromosome from mother star. Iron-alum, haematoxylin. × 2250.

FIG. 30. Chromosome from mother star showing a darker central part corresponding to the position of the chromatin band. Anilin-safranin, gentian-violet. × 2250.

FIG. 31. Chromosome from mother star. Cyanin, erythrosin. × 2250.

FIG. 32. Chromosome from mother star. Cyanin. × 2250.

FIG. 33. Chromosome from mother star, showing mode of division Anilin-safranin, gentian-violet. × 2250.

FIG. 34. Chromosome from mother star, showing mode of division. Anilin-safranin, gentian-violet. × 2250.

FIG. 35. Chromosome from mother star, showing mode of division which appears to be an untwisting of the chromatin loop. Anilin-safranin, gentian-violet. × 2250.

FIG. 36. Macrospore, with nucleus in the mother star stage with numerous micronucleoli in the surrounding cytoplasm, some of which are of a larger size than usual. The chromosomes are ready to be pulled apart. Anilin-safranin, gentian-violet. × 1200.

FIG. 36*a*. Pole of the spindle with centrosphere containing a double centrosome. × 2250.

FIG. 37. Macrospore, with nucleus in the loose daughter skein stage. The numerous micronucleoli in the cytoplasm appear to be in close connection with cytoplasmic radiations surrounding the daughter nuclei. Iron-alum, haematoxylin, erythrosin. × 1200.

FIG. 38. Close daughter skein stage with numerous micronucleoli in the surrounding cytoplasm, cytoplasmic radiations, and fine centrospheres at one pole. Anilin-safranin, gentian-violet. × 1200.

FIG. 39. Close daughter skein stage with micronucleoli in cytoplasm and cytoplasmic radiations. Delafield's haematoxylin. × 1200.

FIG. 40. Close daughter skein with most of the micronucleoli inside of the nuclear area. Anilin-safranin, gentian-violet. × 1200.

FIG. 41. Close daughter skein with most of the micronucleoli inside of nuclear area and the remaining ones crowded around the two nuclei. Anilin-safranin, gentian-violet. × 1200.

FIG. 42. Two-celled embryo sac. Only a few micronucleoli remaining in the cytoplasm. Anilin-safranin, gentian-violet. × 1200.

FIG. 43. Two-celled embryo sac. All the micronucleoli have entered the daughter nuclei, which at this stage have well-defined nuclear membranes. Iron-alum, haematoxylin, orange G. × 1200.

FIG. 44. Section of two-celled embryo sac, showing nucleus in the daughter star stage. Two centrospheres appear at one pole and numerous micronucleoli in the cytoplasm. Anilin-safranin, gentian-violet. × 1200.

FIG. 45. Embryo sac with well marked cytoplasmic radiations, and the micronucleoli crowded around the daughter nuclei. The remains of the first division spindle are still present. Cyanin, erythrosin. × 1200.

FIG. 46. Embryo sac with the two nuclei in the close daughter skein stage. Numerous micronucleoli in the surrounding cytoplasm and the remains of the first division spindle. Anilin-safranin, gentian-violet, × 1200.

FIG. 47. Four-celled embryo sac with all the nuclei in the resting stage, showing parietal position of the nucleoli. No micronucleoli are left in the cytoplasm. Anilin-safranin, gentian-violet. × 1200.

PECULIAR STRUCTURES OCCURRING IN THE POLLEN TUBE OF ZAMIA.

HERBERT J. WEBBER.

(WITH PLATE XL)

THE recent announcement by Hirase[1] of the occurrence of motile spermatozoids in *Ginkgo biloba*, and that by Ikeno[2] of the occurrence of similar organs in *Cycas revoluta*, render any observations on the phenomena occurring in the pollen tube of Zamia, belonging to the related sub-family Zamieæ, of special interest. In cones of *Zamia integrifolia* shortly before fecundation the writer has observed several remarkable structures, which, so far as can be learned, have never been described.

For a considerable period preceding fecundation in Zamia, as in many other gymnosperms, the pollen tube apparatus remains in almost the same condition. no important changes taking place. In this stage the pollen grains, which lie in the pollen chamber at the apex of the nucellus, are found to have germinated and the germ tubes to have reached a length of 1 to 2^{mm}, penetrating into the tissue of the nucellus. The pollen tube is much greater in diameter than the pollen grain which may be clearly distinguished. The vegetative nucleus of the pollen grain, in every case observed, has wandered into the pollen tube and may usually be found near its lower end (*fig. 1*). In the upper end of the pollen tube, near the pollen grain, two cells are uniformly found, one in close connection with the old pollen grain from which it protrudes or is only slightly separated, and the other immediately in front of this in the more swollen portion of the pollen tube (*fig. 1*). The former cell is spherical or slightly

[1] HIRASE, S. — Untersuchung über das Verhalten des Pollens von Ginkgo biloba. Bot. Centralbl. **69**: 33. 14 Ja. 1897.

[2] IKENO, S. — Vorläufige Mittheilung über die Spermatozoiden bei Cycas revoluta. Bot. Centralbl. **69**: 1. 30 D. 1896.

elongated and presents a most singular structure. The nucleus of the original cell evidently divides into two, and one of the daughter nuclei forms within the unbroken *Hautschicht* of the mother cell a new and wholly distinct *Hautschicht* which delimits a cell lying entirely free within the mother cell and surrounded on all sides by a layer of protoplasm of nearly uniform thickness (*figs. 1a* and *2*.) The other daughter nucleus remains free within the *Hautschicht* of the mother cell, but is pressed to one side by the interior cell. The free nucleus usually occupies the side of the mother cell opposite the pollen grain. Both the interior cell and the mother cell are crowded full of small spherical starch grains (*fig 2*). The second cell (*fig. 1 gc*) which is probably to be compared with the generative cell in conifers, is much larger then the first or proximal cell above described, and is provided with two small spherical organs, situated at the opposite ends of the nucleus outside the nuclear wall, that somewhat resemble centrosomes (*fig. 3*). They are exactly spherical and stain deep red with safranin, in the Flemming triple stain process, much as the nucleoli. There is no appreciable difference in size between the spheres in the same cell, but those of different cells frequently vary several micromillimeters in diameter, ranging from 7 to 10 μ. Many of the spheres at this stage appear entirely homogeneous, but in some cases a number of small vacuoles have been observed in the interior (*fig. 4*). Numerous long slender threads of kinoplasm radiate in all directions from the spheres, and in many instances may be seen plainly to extend to the *Hautschicht* with which they frequently appear to unite. The threads of kinoplasm are rather coarse and are plainly visible without staining. With the Flemming triple stain they are colored intensely blue. They appear in many instances to be firmly fastened to the *Hautschicht*, which is drawn in more or less where the thread is attached (*fig. 5*). This seeming indentation may, however, be caused by shrinkage during fixing and staining. The spheres are usually 7 to 10 μ from the nuclear wall, which is in most instances strongly indented on the ends next to the spheres. Threads of kinoplasm run from the spheres to the nuclear wall, which at this stage is apparently

continuous. In most cases observed the spheres occupy positions on exactly opposite sides of the nucleus, but in a few instances they have been found much nearer together, being only about 135° apart. The nuclei which these bodies accompany are at this stage in a resting condition, the contents presenting a fine granular appearance and usually showing foam-structure more or less plainly. The nuclei are usually elliptical and about 80 μ long by 56 μ wide. The large nucleoli (20 to 24 μ in diameter) contain numerous vacuoles. Frequently several other small nucleoli may be observed in a nucleus.

When the activity, which precedes fecundation, begins, the generative cell does not wander down to the distal end of the pollen tube as might be expected from analogy, but instead the proximal end of the tube (the pollen grain end) with the two cells, shown in *fig. 1*, turns directly downward and grows through the apical tissue of the nucellus (*fig. 6*) and may be seen with a hand lens hanging down into the cavity formed above the archegonia. The pollen grain, constituting the extreme end of the now pendent proximal end of the tube, may be plainly seen, the two cells remaining in the same relation to the pollen grain as shown in *fig. 7*. All of the tubes examined show this same structure, indicating that it must be the normal procedure. The first cell which protrudes from the old pollen grain remains in the same condition, or in the most advanced stages yet studied shows indications of disintegration. The generative cell or second cell in the tube, which now lies above the first cell, has usually divided during this extension of the tube, or may be found in some stage of division. The two centrosome-like bodies which accompany each generative cell and during the resting condition, as described above, occupied the poles of the nucleus corresponding to the major axis of the cell and the longitudinal axis of the pollen tube, usually come to lie, during the migration of the cell into the now extended proximal end of the pollen tube, at two opposite points on the equator, the nucleus corresponding to the minor axis of the cell or transversely in the pollen tube. Whether this change in their position is brought about by the migration of the centro-

some-like bodies or by the changed position of the nucleus the writer has not yet been able to surely determine. They have meanwhile greatly increased in size, now measuring usually from 18 to 20 μ in diameter. They now have a clearly distinguishable outer wall of considerable thickness and the contents are evenly and beautifully vacuolate.

When the nuclear spindle is formed these bodies always take up a position directly opposite the polar ends. In the monaster stage of the division (*fig.* 7) the spindle, which occupies a transverse position both to the pollen tube and to the elliptical nucleus, is composed of fine kinoplasmic filaments and seems to be entirely within the still preserved nuclear wall. It is blunt poled, if indeed it is not multipolar, and does not seem to have any kinoplasmic connection with the centrosome-like bodies. In the resting condition of the nucleus the centrosome-like bodies were surrounded by radiating threads of kinoplasm, but no indications of these can now be seen. There is, however, in most cases a slight radial arrangement of the protoplasm immediately bordering the wall. The structure of the bodies has meanwhile undergone considerable change (*figs.* 7, 8). The wall swells up and apparently separates into fragments which in cross-section at this stage show as a broken line. The vacuolated contents meanwhile contract away from the wall, leaving a clear space intervening, which is, however, traversed by a few slender filaments. The body at this stage presents the appearance of disintegration.

When the cell-plate is formed (*fig.* 9) the centrosome-like body is found entirely broken up, the fragments appearing in the cytoplasm as a number of granules mixed with plates or membranes which appear to be fragments of the wall. After the new cell wall is completed and the daughter nuclei have returned to the resting stage, showing a nucleolus, the fragments of the broken-down centrosome-like body present the appearance shown in *fig.* 10. A little cluster of granules appear in the exact location of the body, and the plates, which stain deeply and often appear to be two in number, have separated and moved toward the poles of the daughter nuclei.

The writer has been unable to determine the origin of the centrosome-like bodies described above, and thus cannot be sure as to their nature. In the resting condition of the generative cell (*fig. 3*) they are somewhat similar to the bodies described by Hirase as "attraction spheres"[3] in the pollen cell of *Ginkgo biloba*, but his description and figure differ from what I have seen, in that spherical bodies much larger than the attraction spheres are located between them and the nucleus. No indications of the latter body occur in Zamia. The nucleus, furthermore, is of very different shape from those occurring in my slides of Zamia, and the so-called attraction spheres are apparently much smaller than the similar bodies which I find.

The researches of Farmer,[4] Osterhout,[5] Mottier,[6] and Strasburger[7] have thrown much doubt on the occurrence of centrosomes in the pteridophytes and phanerogams, where the nuclear spindle in its earlier stages is multipolar, at least in the spore or pollen mother cells. In view of these facts their occurrence in Ginkgo and Zamia may well be doubted. The spheres in the generative cell of Zamia resembles centrosomes in that they have the kinoplasmic filament centered upon them during a large part of their existence, and have an important relation to the formation of the spindle, being uniformly located near the poles and always having a definite orientiation with reference to the axis of the spindle. They differ, however, so materially from the centrosomes described by Farmer,[8] Swingle,[9] Strasburger,[10] and others, and

[3] HIRASE, S.— Notes on the attraction sphere in the pollen cells of *Ginkgo biloba* The Botanical Magazine **8**: 359. 1894.

[4] FARMER, J. BRETLAND.— Ueber Kerntheilung in Lilium-Antheren besonders in Bezug auf die Centrosomen-Frage. Flora **80**: 56. ——.

[5] OSTERHOUT, W. J. V.— Ueber Enstehung der karyokinetischen Spindel bei Equisetum. Jahr. f. wissenschaft. Bot. **30**: 159. 1897.

[6] MOTTIER, DAVID M.— Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen. Jahr. f. wissenschaft Bot. **30**: 169. 1897.

[7] STRASBURGER, EDUARD.— Ueber Cytoplasmastructuren, Kern- und Zelltheilung. Jahr. f. wissenschaft. Bot. **30**: 375. 1897.

[8] FARMER, J. BRETLAND.— On spore formation and nuclear division in the Hepaticæ. Annals of Botany **9**: 469. 1895.

from the centrospheres found by Harper[11] in the Ascomycetes, that in the present state of our knowledge they must be considered to be distinct organs. What they are cannot be determined till their origin and functions are better known.

The further highly remarkable history of these organs the writer hopes soon to discuss, together with other details of the fecundation, in a future number of this journal.

U. S. SUBTROPICAL LABORATORY,
EUSTIS, FLORIDA.

NOTE.— As this paper is passing through the press Mr. Webber sends word that he has discovered motile antherozoids in Zamia. They were found in a sugar solution and kept moving for two hours and forty-four minutes.— EDS.

EXPLANATION OF PLATE XL.

Zamia integrifolia Willd.

FIG. 1. Pollen tube growing in the nucellus of the ovary; *pg*, pollen grain; *vn*, vegetative nucleus; *a*, cell protruding from old pollen grain and containing a free interior cell and nucleus; *gc*, generative cell. × 120.

FIG. 2. Proximal cell shown at *a* in preceding figure. × 550.

FIG. 3. Generative cell showing centrosome-like bodies with radiating filaments and kinoplasm. × 450.

FIG. 4. Centrosome-like body during resting stage of nucleus, showing vacuoles. × 1800.

FIG. 5. Centrosome-like body seen from above looking toward the nucleus showing kinoplasmic filaments connected with *Hautschicht*. × 600.

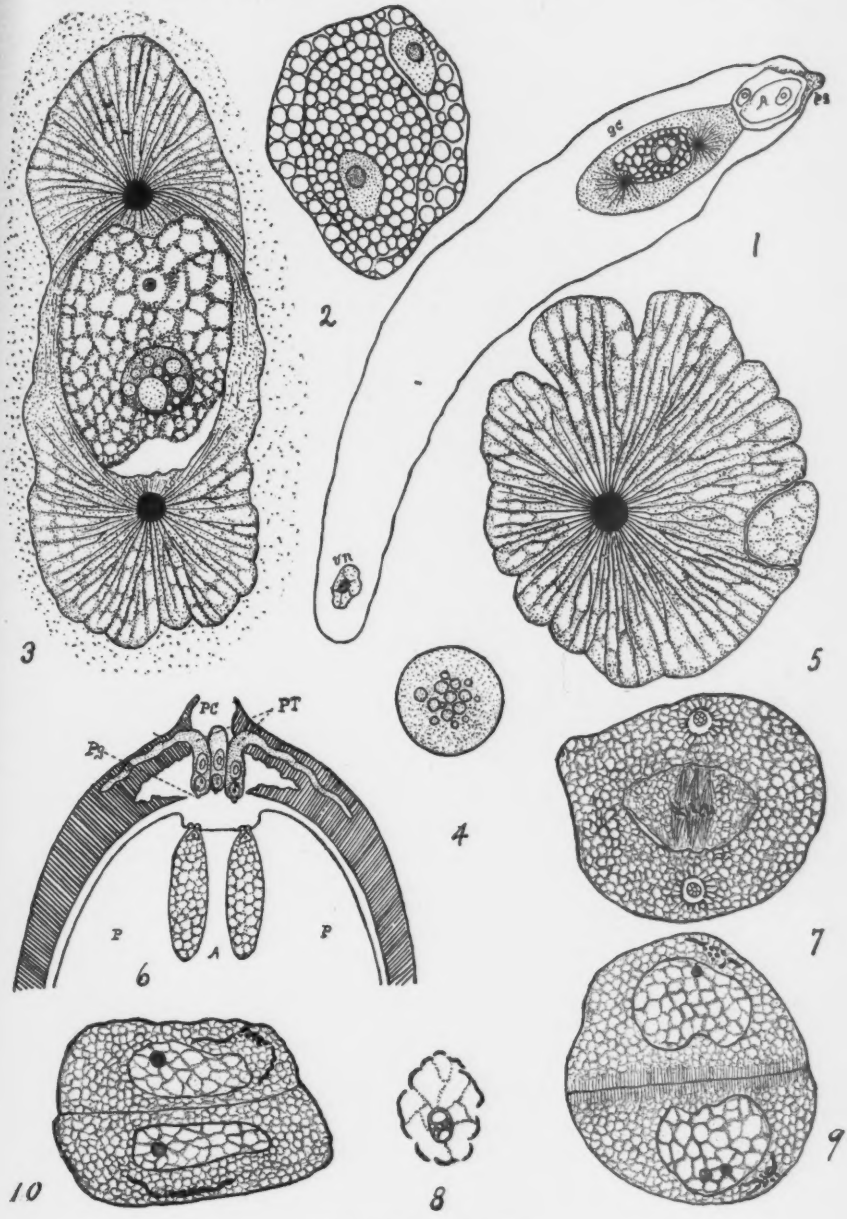
FIG. 6. Diagrammatic outline of the upper end of nucellus, showing the proximal end of the pollen tubes growing down into the cavity just above the archegonia; *p*, prothallus; *a*, archegonia; *pc*, pollen chamber; *pt*, pollen tubes; *pg*, pollen grain.

FIG. 7. Generative cell in monaster stage of division, showing the breaking down of the centrosome-like body and the absence of the radiating kino-

[9] SWINGLE, WALTER T.— Zur Kenntniss der Kern- und Zelltheilung bei den Sphacelariaceen. Jahr. wissenschaft. Bot. **30**: 297. 1897.

[10] STRASBURGER, EDUARD.— Kerntheilung und Befruchtung bei Fucus. Jahr. f. wissenschaft. Bot. **30**: 351. 1897.

[11] HARPER, R. A.— Kerntheilung und freie Zellbildung in Ascus. Jahr. wissenschaft. Bot. **30**: 249. 1897.



WEBBER on ZAMIA.

plasmic filaments which surrounded it in the resting condition of the cell. × 200.

FIG. 8. Centrosome-like body from cell illustrated in *fig.* 7. × 600.

FIG. 9. Generative cell in late stage of division, showing the fragments of the centrosome-like body. × 200.

FIG. 10. Generative cell after division with the fragments of the centrosome-like bodies showing the plates or membranes approaching the poles of the daughter nuclei. × 200.

BRIEFER ARTICLES.

CURIOUS LEAVES.

THE leaves of trees not infrequently present sports or deviations from the ordinary types characteristic of the species, and occasionally these sports are of botanical interest. A few collected near Dayton, Ohio, during the autumn months of last year, are here figured.

A twig from an American elm has a terminal leaf with its basal margins grown together, forming a short funnel-like cavity, with a very oblique upper rim. The third last leaf presents a similar funnel-like cavity at its base, but the leaf blade is supplied with two midribs and leaf tips. The second last leaf shows two midribs and leaf tips, but the basal margins of the leaf blade have remained separate (*figs. 1, 2*).

Near the tip of a young shoot from a stump of the white ash were small leaves with the usual pointed leaflets. In the figure (*fig. 3*) all but one of these leaves have been removed. The next lower pair of leaves, however, has most of the leaflets notched at the tip. The most interesting one of these is the terminal leaflet of the right hand leaf, which, although slightly notched at the tip, bears itself a second terminal leaflet pointed in the usual way.

A third sport occurred in the leaves of some of the minor branches of the ordinary dogwood (*fig. 4*). Here the leaves instead of being pointed in the usual way have assumed the form of the involucral bracts which are taken by the unwary for the petals of the flower. Their color, however, was green.

A study of numerous sports of this kind has developed the following facts: In trees with opposite leaves it rarely happens that only one leaf of a pair deviates from the type form. Usually both leaves show the same, or at least similar variations. In the case of both trees with opposite and those with alternate leaves several successive nodes usually show either the same or similar variations. Several of the neighboring branches, and even branches of neighboring trees, may present the same variation, but in those cases the variations seem

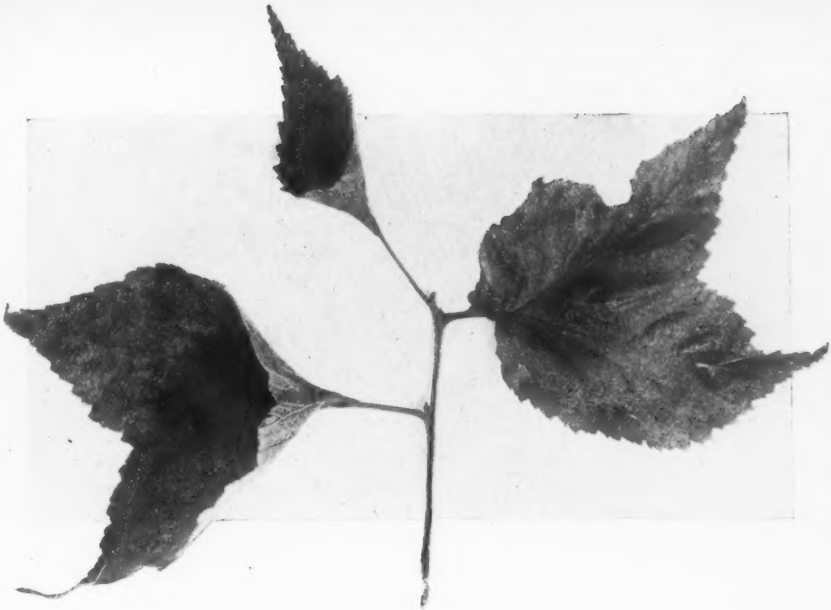


FIG. 1.

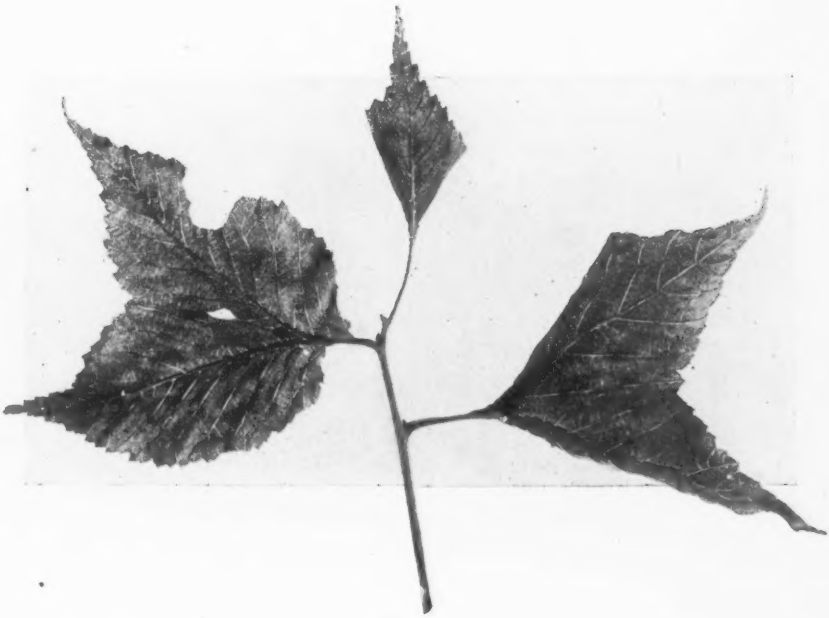


FIG. 2.

to have been very nearly synchronous, judging by the position of the sports along that part of the branches formed during the year.

The appearance of similar sports along successive internodes has led to the belief that each node and its appendages may be considered a plant unit of which the next node is an offspring. In this sense the successive appearance of similar sports along the same branch may be looked upon as a case of heredity. The simultaneous appearance of similar sports in neighboring branches or trees, however, indicates that this explanation is not sufficient. In order to arrive at a proper basis for the interpretation of these facts it is necessary to know the life history of the plant. For instance, at what time of the year does the elm leaf begin to differentiate its cells into those which shall become a part of the midrib, and those which shall not? At what time of the year do the leaf blades begin their development? When are the forms of the tips of ash and dogwood leaves determined? Is it not during the vicissitudes of autumn, the winter months, and very early spring? It is believed that a further study of this subject will indicate that freaks of a marked kind often accompany very marked meteorological irregularities, and that there is often a vital connection between the two.

In a similar manner it has been noticed that ash twigs with three leaves at a node are formed more commonly on the young shoots which spring up from the stump of the tree the first year after the tree has been cut down. In other words, while we know as yet very little about the conditions which give rise to sports, it is beginning to be evident that even these evanescent freaks of nature stand in relationship to other conditions as cause and effect; that they are sporadic attempts on the part of nature to accommodate itself to variant conditions at present ill understood.

These sports belong, perhaps, to the same order as those peculiar sports among gold fish so popular with the Chinese, gold fish with tripartite tail, caused, it is said, by shaking up the eggs of fishes violently before they are hatched.—AUG. F. FOERSTE, *Dayton, Ohio.*

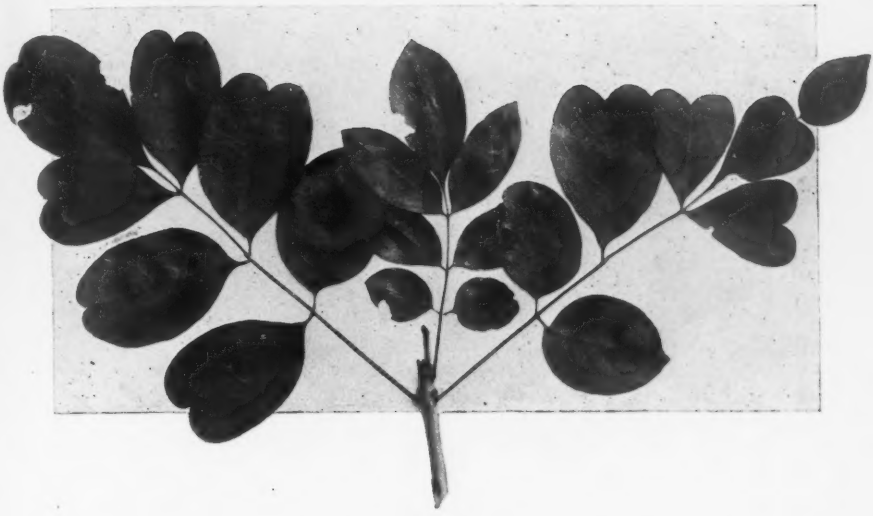


FIG. 3.

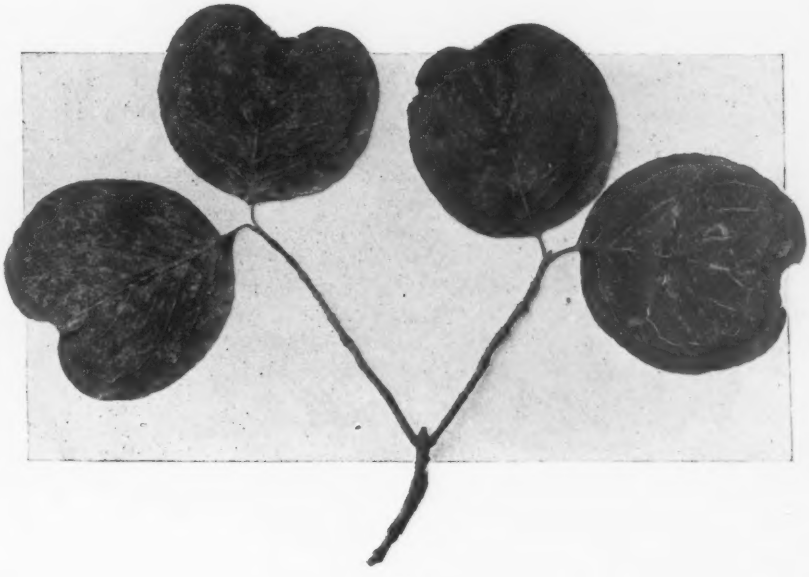


FIG. 4.

OPEN LETTERS.

SPECIES OF BOTRYCHIUM.

To the Editors of the Botanical Gazette:—In the second edition of *Gray's Manual of Botany* (1856), and continued in the third and fourth editions, under the species *Botrychium Virginicum* occurs this remarkable statement:

"Var.? *simplex* (*B. simplex*, Hitch.) appears to be a remarkably depauperate state of this, only 2′–5′ high, the sterile frond reduced to a single short-stalked division, and simply or doubly pinnatifid," etc.

I cite the above to show that the practice of reduction of distinct species of Botrychium is one that has long been followed beyond the river Charles.

There are to my knowledge three general accounts of the genus Botrychium that have appeared within the past thirty years, and as they differ somewhat widely I reproduce the disposition of species in each case. In the last column a double star indicates the species accredited to our territory.

Milde, 1868, 1869, 1870[1]	Baker, 1874; reiterated, 1891[2]	Prantl, 1884[3]
1. B. Lunaria Sw.	1. B. simplex Hitch.	1. B. Lunaria Sw.**
2. B. crassinervium Rupr.		
3. B. boreale Milde.	2. B. rutaceum Sw.	2. B. boreale Milde**
4. B. matricariaefolium A. Br.		3. B. lanceolatum Angs**
5. B. lanceolatum Angs.		4. B. matricariaefolium A. Br.**
6. B. simplex Hitch.	3. B. Lunaria Sw.	5. B. simplex Hitch.**
7. B. ternatum Sw.	4. B. ternatum Sw.	6. B. ternatum Sw.
		7. B. daucifolium Wall.
		8. B. subbifoliatum Brack.
8. B. daucifolium Wall.	5. B. daucifolium Wall.	9. B. australe R. Br.
		10. B. silaifolium Presl*
		11. B. obliquum Willd**
		12. B. lunarioides Sw **
		13 B. rutifolium A. Br.
9. B. lanuginosum Wall.	6. B. Virginianum Sw.	14. B. lanuginosum Wall.
10. B. Virginianum Sw.		15. B. Virginianum Sw.**

The first record of the *ternatum* group from America appears to have been that of Lamarck's species 1797 (*Osmunda biternata*), which was re-described by Michaux in 1803 (*Botrypus lunarioides*) under another name. In Pursh's Flora (1814) there was introduced a confusion which continued until the modification of Milde's arrangement which appeared in the sixth edition of *Gray's Manual of Botany*. That Milde fell into Pursh's error of confusing various Atlantic forms under Michaux's name is perfectly evident from his text:—(1) Milde's description of *lunarioides* is generalized and

[1] Verhandl. k. k. zool. bot. Ges. Wien **18**: 507–516; **19**: 55–190; **20**: 999–1002.

[2] Synopsis Filicum 447, 448. 1874; Ann. Bot. **5**: 500. 1891.

[3] Jahrb. des kön. bot. Gartens, Berlin **3**: 297–350. 1884.

indefinite, and while it may well cover a great variety of forms it does not at all delimit the very distinct *Botrychium biternatum* (Lam.); (2) from his quotation, or rather translation, of the range given by Pursh: "Auf Triften und in lichten Wäldern von New York bis Carolina (Pursch);" (3) from his later citation of additional localities for the plant from Lake Superior (Macoun) and Montreal (Watt) which, as all northern forms were at that time confused under the name *lunarioides*, Milde evidently either quoted from some published list or may have received specimens and quoted the current labels; if the latter more the pity. There is nothing more certain than that Milde did not at all understand the very unique character of the exclusively southern plant, and Mr. Davenport's statement, "I cannot believe it possible for him to have been mistaken in any specimens coming under his observation," reminds one more of sentimental hero worship than of a sincere attempt to know the truth. The citation of "authority" and "the opinion of the fathers" is as obsolete in botany as it is elsewhere. It does not surprise me that Mr. Davenport has sought in vain to find anything approaching *lunarioides* in Professor Macoun's collections. The collections of the past sixty years in northern areas has failed to bring it to light, and it is not likely that it exists.

Mr. Davenport's paper well illustrates the dilemma he is in in attempting to refer accurately to any one *thing* in his various references to *Botrychium ternatum*. At one time he is talking of one thing, and in a later sentence of another entirely different. This aggregate consists of several very distinct things, *i. e.*, distinct species, and to continue to refer to the aggregate as *one* is both confusing and unscientific.

In Mr. Davenport's zeal to reduce the species to varietal rank he seemed to overlook my statement that "the true *Botrychium ternatum* is comparatively common in central Alabama and produces its spores late in the season (August to October), the same as it does farther north[4]," and his effort to extend the season of the two species so that their extremes will not seem so widely separate must excite a smile among persons thoroughly familiar with the plants in the field. So far as I can see, the only point that Mr. Davenport has established is that the bud of some specimens of *Botrychium biternatum* is somewhat hairy (if, indeed, he is sure of his specimens, some of which I regard as very doubtfully true *biternatum*), and I fully agree with him in regarding the bud character in the genus, which he has formerly made so much of, as a somewhat unreliable one. I still regard the form which Lamarck first described as *Osmunda biternata* as distinct a species of Botrychium as exists in the country. I am, however, open to evidence, and request that during the present season observers in all parts of the country note the variations in this interesting group and send me material illustrating all the variations in their respective localities.

[4] BOT. GAZ., **22**: 408. N. 1896.

So long as my own field observations on Botrychium were confined to central New York and New England, I regarded all the forms that there appear as running into each other and so discarded the "varieties" as trivial. I had never, indeed, until last season seen in the field the genuine form that Sprengel long ago described as *Botrychium dissectum*, a type that sixteen years of collecting in New England, and a large array of material from all parts of that territory, has not revealed as a New England form. Mr. Davenport's statement that it is a common New England form only reveals the fact that he is confusing with it a very different plant which *is* common in New England and elsewhere, but has little in common with the genuine *dissectum*. Had I experienced the misfortune to have my field work confined to eastern Massachusetts I might even yet be holding Mr. Davenport's ultra conservative notions. As it is, I believe now that while the evidence is not all in, the present indications are that Prantl's arrangement of the American species is far more logical than any other arrangement that has yet appeared, and that we have in America in the *ternatum* group a series of species even more distinct when rightly understood than the species of that other closely allied group that Baker so unceremoniously and illogically places under the aggregate "*Botrychium rutaceum* Swz."[5] I am anticipating the pleasure of soon going over the evidence at Kew and the types at Paris, and shall hope that a still wider range of data will help us to arrive at a better understanding of the genus.

It is unnecessary to discuss further Mr. Davenport's position, for his mind was fully made up in advance, since he wrote me some time ago that "Milde had said the last word on Botrychium, as though any problem of taxonomy could be settled by an appeal to "authority," and before the evidence was all in.— LUCIEN M. UNDERWOOD, *Columbia University*.

COLOR IN PLANTS.

To the Editors of the Botanical Gazette:—In your issue of January 1897 there is a notice of Professor Wittrock's studies on the history and origin of the garden pansy, at the conclusion of which is the following pregnant sentence, viz.: "If the pollinating insects prove to be color-blind, as is claimed now by certain physiologists, the yellow eye, as well as all floral coloration, will need a new explanation."

I venture to point out that such a new explanation is suggested in an article entitled "Organic color," which appeared in *Science*, June 16, 1893, published in New York. If any scientist who feels interested in the subject would consider and criticise that paper a usetul discussion might ensue.—F. T. MOTT, *Crescent House, Leicester, England.*

[5] It is worth noting that recent European monographers follow Prantl in separating the European species (*B. rutifolium*) from the *ternatum* muddle in which Milde left it. *Cf., e. g.*, Luerssen in Rabenh. Krypt. Flora **3**: 582–588.

CURRENT LITERATURE.

BOOK REVIEWS.

Experimental morphology.[1]

It is with pleasure that we welcome a text-book in this comparatively new field of biology. While embryology is investigating the problem, *how* adult forms are produced, it is the new school of physiological morphology that deals with the question "*why* does an organism develop as it does?" And it is with this question of such great importance to physiologists and morphologists alike that this work occupies itself.

This first part is devoted to those processes which are characteristic of all living protoplasm, and it is quite needless to say that both plants and animals are included in its range.

One of the characteristics of the book is the stress laid upon quantitative measurements of agents and effects. No better opening sentences could have been selected than those of Jaeger and Jevon:

> Die morphologische Betrachtung setzt also eine genaue chemische und physikalische Kenntniss 1. des betreffenden Körper selbst und 2. aller der bei seiner Entstehung auf ihn einwirkenden Stoffe und Körper voraus.

"There can be little doubt, indeed, that every science as it progresses will become gradually more and more quantitative."

The book is divided into nine chapters. The first deals with the action of chemical agents upon protoplasm, and it is here especially that the great value of exact quantitative work becomes apparent. The third section, devoted to chemotaxis (chematropism), is doubtless one of the most attractive in the whole work. Pfeffer's classical experiments are quite fully related.

From the second to the eighth chapters the author treats of the effects of (1) moisture, (2) density of medium, (3) molar agents, (4) gravity, (5) electricity, (6) light, and (7) heat. In general it may be said that a fair historical review is given of the literature on these subjects. In a few cases, it is our opinion that better illustrative experiments could have been chosen. In some cases too much space is given to the Protista, to the exclusion of the Metazoa. This is especially so in the section on stereotaxis (stereotropism), where only a bare reference is made to the stereotaxis of multicellular forms

[1] DAVENPORT, C. B., Experimental morphology, Part I. 8vo. pp. xiv + 280 New York: The Macmillan Company. $2.60.

Doubtful and perplexing examples of Protista and spermatozoa are presented, while such clear and striking examples as are afforded by the experiments of Dr. Loeb on the moth Amphipyra are not mentioned. The same objection holds with still greater force in regard to the chapter on geotaxis.

Another thing which we regret is that in a text-book of biology, which evidently bases all biological phenomena upon the chemistry and physics of the organism and its environment, such metaphysical terms as photophil, photophob, lovers of dark, etc., are used. The same applies to the terms so frequently used, "adaptation," "advantageous to the organism." If by adaptation is meant no more than if we were to say that the photographic plate is adapted to the action of the light, the term is misleading. If, however, by such terms more is understood, it brings physiology back to the realm of metaphysics, a result contrary to the general tendency of the book.

We are glad the author, even at the risk of becoming wearisome to the ordinary reader, goes quite extensively into the physics and chemistry of such subjects as light and solutions. The fuller description of methods will certainly be highly appreciated by the student. Indeed, seeing how much in biological investigation depends upon methods, we could almost wish the author had been still more elaborate in this respect.

In general, the subject matter is well presented. Relatively much space is given to the facts and little to conflicting theories. The style is clear and concise. The bibliography will be of great use to the investigating student. The spirit of the first part is such that we shall look with impatience for the other three parts on growth, cell-division, and differentiation. The author has certainly done a great service to the student of biology in the careful collecting of the numerous researches in the field of experimental morphology, and we doubt not that the book will prove of immense value as a text, and as a stimulus to further and more thorough investigation.—W. D. ZOETHOUT.

Vegetables under glass.

THE last issue of the "Gardencraft Series"[2] deals with the forcing of vegetables. It is precisely what its subtitle indicates: a manual of the cultivation of vegetables in glass houses. It gives explicit directions for the construction and management of forcing houses, enumerates the vegetables commonly grown or capable of being grown in such houses, and gives detailed instructions for the growing of each. These instructions are based upon results actually worked out by the author and others. While the author is convinced that the forcing of vegetables is "bound to open up great possibilities for the future," he is conservative in advising beginners to undertake

[2] BAILEY, L. H., The forcing book; a manual of the cultivation of vegetables in glass houses. 12mo., pp. xiv+266, figs. 88. New York: The Macmillan Company, 1897.

the work, and carefully points out the difficulties that must be surmounted. Unquestionably this is the most comprehensive and valuable book that has thus far been published on the subject, and no one who is engaged in the forcing of vegetables, or who contemplates engaging in it, can afford to be without it.

A considerable part of the subject matter of this book has already been published by the author or his assistants through the bulletins of Cornell University, and the author has quoted rather freely from other sources. But the parts are so well adjusted, and so well supplemented by the author's hitherto unpublished experiences and observations that the somewhat fragmentary structure of the book does not appear, and the freshness, clearness and grace that characterize all of Professor Bailey's writings abound throughout. If his sentences are sometimes less polished than we might expect from so learned a writer, the intensity of their expression and the fertility of the thoughts they convey always render them most pleasant and profitable reading.—E. S. GROFF.

Monographia Cactacearum.[3]

Botanists and gardeners everywhere will greet with pleasure Professor Schumann's *Monographia Cactacearum*, the first part of which has just appeared. An inspection of this justifies the assertion that expectation will not be disappointed; for the work promises to satisfy in a large measure the long felt needs not only of botanists, but also of cactus growers generally, amateur and professional. The author has studied the group during the greater part of eight years, visiting the principal botanic gardens of Europe, constantly examining growing plants in all stages, and bringing together in Berlin an unsurpassed collection of living and dried material. Certainly the Botanic Garden in Berlin, with its cactus prestige of nearly a century, furnishes rare opportunities for such a comprehensive study as Professor Schumann has undertaken, for in this, as in no other family of plants, the element of culture tradition enters as an exceedingly important factor. It happens in numerous species of all genera that existing individuals can with absolute certainty be referred back through years of culture to their originals, constituting a thread of identity which would otherwise long since have been quite obliterated. It has thus been possible in the present work to rescue many of the older species from oblivion, not, however, without that ever present element of uncertainty that arises from the instability of vegetative characters so prevalent in the family. Furthermore, as guiding spirit in the Berlin Gesellschaft der Kakteenfreunde, and as editor of the *Monatschrift für Kakteenkunde*, the author has long been in close communication with an army

[3] SCHUMANN, KARL.—Gesamtbeschreibung der Kakteen (monographia Cactacearum). Part I, large 8vo, with 100 text cuts. Neudamm: J. Neumann. *M.* 2.

of collectors and cactus growers, the importance of whose cooperation in this knotty group can not be overestimated. The work will appear in ten fascicles at intervals of two months, constituting when complete a handsome royal octavo volume of 600 pages or more.

With the exception of a rather comprehensive introduction devoted to general morphology and geographic distribution, the work is purely systematic, with short, excellent, clear cut descriptions. In his chapter on distribution the author brings up the old but interesting question of the origin of old world forms. The original home of *Opuntia vulgaris* remains unsettled. The widespread occurrence of Rhipsalis in tropical Africa is logically accounted for through the instrumentality of bird migration, the mucilaginous juice of the berry suggesting the possibility of an occasional seed clinging to feathers for a considerable period. The "author's index" presents a novel feature in the form of personal or biographical comment, furnishing to cactus lovers an interesting and useful compendium of information.

The family is subdivided as follows:

I. Subfamily CEREOIDEÆ.

Tribe 1. *Echinocacteæ.*—Cereus, Pilocereus, Cephalocereus, Phyllocactus, Epiphyllum, Echinopsis, Echinocereus, Echinocactus, Melocactus, Leuchtenbergia.

Tribe 2. *Mamillariæ.*—Mamillaria, Pelecyphora, Aniocarpus.

Tribe 3. *Rhipsalideæ.*—Pfeiffera, Hariota, Rhipsalis.

II. Subfamily OPUNTIOIDEÆ.

Tribe 4. *Opuntiæ.*—Opuntia, Nopalea, Pterocactus.

III. Subfamily PEIRESKIOIDEÆ.

Tribe 5. *Peireskieæ.*—Peireskia.

It will be noticed from the above that twenty genera are recognized, of which one is new. *Cephalocereus* (Pfeiff.) em. K. Sch. has for its typical representative our Mexican "old man cactus," *Cephalocereus senilis. Pterocactus* K. Sch., from Argentina, is a most remarkable representative of the Opuntioideæ, being distinguished not only from the other genera of its tribe, but from all other Cactaceæ, by its circumscissile dehiscent capsule and broadly winged seed.

Further particulars, so far as they are of general systematic interest, will be mentioned from time to time in this journal as the successive parts appear. —E. B. ULINE.

Septal nectaries.

Though numerous studies have been made of the nectar glands of the ovarian septa of monocotyledons, first described in 1855 by Brongniart, Schniewand-Thies[4] has submitted them to a comparative examination as to

[4] J. SCHNIEWAND-THIES.—Beiträge zur Kenntnis der Septalnectarien. Pp. 87, *pl. 12.* Gustav Fischer: Jena. 1897. *M.* 15.

their morphology and the behavior of their cell contents during the period of greatest activity.

From the simple external nectary of *Tofieldia palustris*, in which the secretion is effected by the epidermal cells of the entire outer wall of the ovary, appearing first as a subcuticular accumulation, and the slightly more specialized nectary of *T. calyculata*, where the similarly subcuticular secretion is limited to the epidermal cells of the septal grooves, a gradually increasing complexity is traced to the Bromeliaceæ, which have complex branched glands deeply seated in the tissues of the ovary — though they really represent gaps between the partially fused carpels, so that they are likewise lined by epidermal cells, which, however, have a well-developed subjacent secreting parenchyma — and the conclusion is reached that the simplest glands belong to genera or species which stand lowest in the systematic classification.

The results of the morphological study are summarized in the tabulation of septal nectaries under the following seven groups, in which the increasing size and complexity of the glands is accompanied by a corresponding development of the vascular system of the ovarian walls:

A. *Ovary superior.*

1. Simple external nectaries (Tofieldia).
2. Double nectaries, in which each outer nectariferous groove passes at top into a septal fissure which is commonly more active (Scilla, Yucca).
3. Inner nectaries, having the general structure of the preceding, but the inner clefts only active (Asparagus, Allium).

B. *Ovary partly inferior.*

4. Mostly double nectaries, with the inner cleft increased in surface by being folded, and the upper part sometimes reduced to a mere duct (Phormium, Hemerocallis).

C. *Ovary inferior.*

5. Double nectaries, the inner clefts more or less complicated (Beschorneria, Crocus).
6. Inner nectaries, opening at top of the ovary (Agave), or in ducts near its middle (Bilbergia).

D. *Ovary superior or partly inferior.*

7. Nectary consisting of three outer and three inner septal fissures, and three inner clefts pertaining to the sutures of the several carpels, into the cavities of which and the stylar canal they run, above (Pitcairnia, Dyckia, Vriesea).

The general conclusion is reached that the cell contents of the secreting tissues are actively concerned in (1) the storage of carbohydrates and albuminoids which are subsequently used in the formation of nectar; (2) supplying the necessary water; (3) converting the accumulated materials into nectar; (4) the spontaneous passage of the nectar outwards.

As a rule, to which Hemerocallis offers exceptions, the nuclei of the secreting tissues become disorganized and dissolve earlier than those of the

adjacent parenchyma, though at first richer in chromatin. In most active nectaries they are said to have a predilection for blue stain, though in some cases they have been found, either at first or throughout, to take the red stain by preference.

The behavior of the chromatin and nucleolar contents of the nuclei during activity of the gland appears to differ greatly in different plants, but in general one or both diminishes noticeably. Nuclei becomes deformed and lobed or even fragmented, send out pseudopodia like prolongations to the ends of which plasma threads become attached, or the nuclear membrane is absorbed and the nuclear material diffuses in the cytoplasm, which itself gradually diminishes or even disappears; meantime the starch accumulation in the vicinity of the nectary is used up, though some reserve starch is often brought into this tissue later.

In the main the descriptions of structure are clearly written, and the plates are all excellently drawn and reproduced. If any fault were to be found with the paper it would be that the student of a given genus or species is confused by the division of the work and plates into separate sections, each of which contains partial studies of a number of species, which, in the absence of a general index, cannot well be united by the reader.—W. T.

Physiological plant anatomy.[5]

THE first edition of this book appeared in 1884. In its preface the author explains that his endeavor was to make plain the connection between anatomical structure and physiological performance. In the preface of the present edition the author states that the subject has made such progress that the book must be much enlarged. The plan and manner of arrangement has not been materially changed, about 150 pages and 95 illustrations having been added. The same number of chapters is retained, although an entirely new one on the apparatus for special functions is added; the two on normal and abnormal growth in thickness being here combined. An introduction and a discussion of the plant cell is added to the first chapter. In short, while still holding to the same object as before, the book is enlarged and improved in every chapter. The author says of the new edition:

> It is no longer the elements of physiological anatomy, but a veritable text-book; not a hand book or cyclopedia, because a great deal of relevant matter has been omitted. All the research that serves only to broaden our knowledge is omitted, and only that used which has deepened it.

One of the most helpful additions to the new edition is the introduction, where the author states clearly and fully the meaning of the expression "physiological plant anatomy." In doing this he gives a new significance to

[5] HABERLANDT, DR. G.—Physiologische Pflanzenanatomie, Wilhelm Engelmann: Leipzig. 1896.

the word anatomy, a meaning which is necessary to the very conception of physiological anatomy. He defines the scope of morphology as including the outer and inner structure. The inner structure includes histology and anatomy; as far as the inner structure is confined to the elements of the separate tissues, it is histology; when we go farther and consider the tissues in relation to their position and arrangement in the plant, it is anatomy. This brings out clearly the principle upon which physiological anatomy is based, that is, the connection between function and form, when form is limited to certain tissues and combinations of tissues inside the plant. These tissues and combinations of tissues are therefore to be considered as organs in the real sense of the term, and not simply as the component parts of the plant.

Another prominent feature of this book is the copious notes or references at the end of each chapter. Here are discussed in detail those points of the text which may be considered debatable, and all the authorities on such questions are cited. For example, Strasburger maintains that the anatomical-physiological idea is not a part of morphology, but must be considered as belonging to physiology. He expressly states that morphology is based upon phylogenetic principles only and has nothing whatever to do with the idea of function. This Haberlandt considers a too narrow conception of morphology. He makes phylogenetic morphology only a branch of the whole subject, saying that as we characterize a tissue or tissue system, like the palisade tissue or the skeleton system, by its morphological qualities as well as by its physiological performance, we must admit that such tissues and systems may be treated from a morphological standpoint.

He further claims that this method of classification of tissues is the broadest of all and the only one based on purely scientific principles, because it considers the plant as an individual organism consisting of elementary organs by virtue of which it is enabled to carry on a series of life processes.

Other methods of classification may be used, but they must be carried out in a manner consistent with the principles upon which they are founded. But a method purely didactic, which aims only to form a convenient basis for a general view of the different tissues cannot be called scientific. These clearly expressed statements of the two opposing views concerning the nature and scope of morphology are of special interest at the present time, as they serve to define the principles held by physiological anatomists, as contrasted with modern morphologists. For example, Strasburger maintains that phylogeny is the only basis of morphology, and that the only way to determine morphological characteristics is to show that one form has been derived from another. But Haberlandt claims that we do find certain morphological characteristics in any cell complex which is sufficiently unified to be called an organ, or to have a separate individual function. Therefore, we are justified in disregarding the notion of phylogeny, and in constructing a set of tissue systems from purely ontogenetic considerations, and of these

we may choose only those pertaining to function. On the other hand, we are also justified in classifying the tissue systems according to phylogenetic principles, but this is not the most logical method.

These statements are also of importance in showing the advance actually made in this subject during the past twelve years. Twelve years ago botanists were unwilling to relinquish the idea of the unity of the so-called fibrovascular bundle, and still regarded the views of Schwendener on the primary function of the thick walled cells of the monocotyledons with a certain degree of suspicion. All this is changed, and in all our recent text-books in which this subject is treated, the complete bundle is spoken of as consisting of stereom and mestom, and the elements of the former are described as mechanical or supporting cells. Thus the truth of the first principle upon which physiological anatomy was founded is fully recognized and admitted by all.

The first chapter of Haberlandt's book contains a full and modern treatment of the typical plant cell, a description of plant tissue, and a classification of tissue systems according to the anatomical physiological principle.

The second chapter treats of tissues in general and the relation of meristems to lasting tissues, and here we find a departure from the interpretation usually given to the developmental processes of the apical region. In all higher plants, at a greater or less distance from the apex, the uniform primary meristem cells differentiate into several distinct building tissues or meristems. These at first show no difference of outer or inner meristems, except the mere topographical one and the histological difference between strand-tissue and ground parenchyma. Regarding the function of the lasting tissue which is to be developed they give no hint. These primary meristems are not necessarily connected with any special kind of apical growth as regards their origin or arrangement. They may be found in plants having only a single apical cell, also in those with several initial cells. That is, three distinct meristems, which Haberlandt names protoderm, procambium, and ground tissue, may be found in the apical regions of plants from the moss upward. He states that since the time of Hanstein's investigations many plants have been examined which do not show a separation into the three distinct meristems, plerome, periblem and dermatogen, and that the manner of apical growth in the phanerogams is subject to too great variation to allow the selection of a single type as furnishing a general law.

The succeeding chapters treat of the eight tissue systems. The plan in each case is to explain first the advantages which the plant derives from the system under consideration, and then to give a clear and full description of its elements and their relations to other parts of the plant. A short paragraph in each chapter is devoted to the system as it occurs in the thallophytes, and its developmental history forms the concluding paragraphs of each chapter, except in the case of the absorption system where such an exposition

is unnecessary. The chapters on the absorption and assimilation systems are considerably enlarged, the former by a paragraph on the absorption of water by the hairs of foliage leaves. The chapter on the conducting system is also much enlarged, the author giving an exposition of the theories of water conduction and explaining the present status of our knowledge of this difficult and perplexing subject. Another section of the same chapter illustrates the author's idea of the dependence of form upon function, that is, that organs are called into existence by some special need of the plant. In this connection he gives an hypothesis concerning the probable manner of development of the different kinds of bundles.

The chapter on apparatus for special purposes contains a description of the various means of plant motion. The passive organs are the flying and swimming tissues which are fully described and illustrated. The active tissues are described as hygroscopic and living, the latter including those through which movements are caused by outer stimuli. The tissues supposed to receive stimuli and those designed only for their conduction are fully treated.

In style the book is exceedingly clear and attractive, and the principles upon which its method of treatment rests are now admitted by all. It is questioned by some, however, whether this classification of tissue systems should be substituted for the older and simpler one which is now in general use. The objections are that it presupposes at least a partial knowledge of the tissues, and that it is too extended to find a place in a text-book on general botany. Both of these objections have more weight perhaps in this country than in Germany. It is also true that the simpler method is far more practical for students of pharmacy and medicine, and for any others who wish only a general view of this branch of botany. In view of these considerations it would seem wiser for us, at least, to precede such a view as Haberlandt presents by a general view based simply upon didactic principles.—EMILY L. GREGORY.

NOTES FOR STUDENTS.

THE ACTION of the yeast cell during alcoholic fermentation has always been a difficult matter for the physiologist to explain. Most writers for the last twenty-five years have considered fermentation a specific form of protoplasmic activity, possessed by certain species of lower plants in a highly developed form. The view of Traube (1858) and of Hoppe-Seyler, ascribing fermentative action to an albuminoid compound secreted by the yeast cell, allied in its nature to the enzymes, has never found favor with botanists. Nägeli in his carefully considered theory of fermentation (1879) pointed out very important differences between the behavior of so-called organized and unorganized ferments, and laid particular stress upon the fact that it had

been found impossible to separate any substance from yeast or other organized ferment that would produce an alcoholic fermentation independent of living protoplasm. Sachs has also elaborated the same opinion. The problem has been further complicated by the assumption by Pfeffer, Wiesner, Noll, and others, that alcoholic fermentation is identical with intramolecular breathing, and therefore through a series of gradations with normal breathing.

A discovery which promises to be of great importance in this connection was announced by Dr. Eduard Buchner[6] of Tübingen in a preliminary communication to the German Chemical Society on January 11. He has separated a nitrogenous compound from yeast which produces a vigorous and characteristic alcoholic fermentation without the presence of yeast or bacteria cells, *i. e.*, independent of living protoplasm.

The method pursued in separating the ferment was as follows. A thousand grams of pure compressed yeast were mixed with an equal weight of quartz sand and 250 grams of diatomaceous earth and the whole ground together until the mass became moist and plastic. This was mixed with 100^{cc} of water and then subjected to a pressure of four to five hundred atmospheres in a hydraulic press. By this means about 300^{cc} of liquid was secured. The remaining cake was broken up and mixed with 100^{cc} of water, and being pressed as before yielded about 150^{cc} additional liquid, which was added to the first. The liquid, being somewhat turbid, was shaken up with 4 grams of diatomaceous earth and filtered through paper several times.

A clear liquid was thus obtained having a specific gravity at 17°C. of 1.0416 and yielding a large percentage of dry substance. By microscopic and bacteriological tests it was found to be absolutely free from yeast cells, and almost or quite free from all other germs.

When this extract is added to an equal amount of a strong solution of either cane, grape, or invert sugar, a vigorous fermentation starts up after a quarter of an hour to an hour and continues for days. There is no action, however, with lactose or mannite, substances which do not ferment with living yeast. The fermentation is not prevented by the presence of chloroform. The liquid lost its fermentative action in a low temperature after five days, but in presence of cane sugar kept it for two weeks under otherwise the same conditions. When heated to 50°C. a flocculent precipitate forms and the power of fermentation is lost. When precipitated with alcohol and dried over sulfuric acid it became inactive. The active principle appears to be incapable of dialysis, but its exact behavior in this regard has not yet been determined.

The author believes that he has demonstrated that alcoholic fermentation is a chemical change, carried on independent of living protoplasm by means of a characteristic proteid substance, for which he proposes the name *zymase*.

[6] BUCHNER, EDUARD: Alkoholische Gärung ohne Hefezellen. Ber. d. deutsch. chem. Ges. 30: 117–124. 1897.

This substance appears to have characters sufficiently different from the enzyms to entitle it to be placed in a distinct class of compounds. It seems probable, although not yet proven, that the yeast cell excretes the zymase into the surrounding liquid, and that the fermentation takes place outside of, and not within the living cell.

From the results so far obtained it appears safe to conclude that alcoholic fermentation is brought about by a non-living substance allied to the enzyms, and that the process is entirely distinct from both intramolecular and normal breathing. Further research along this line will undoubtedly bring to light other important discoveries.—J. C. A.

CAPTAIN HENRY D'ALBERTIS in 1893 equipped the "Corsaro," and on June 3 sailed from Genoa, following as closely as possible the course of Columbus in his voyage of discovery, and on July 20 reached the island of Guanahani,[7] called also San Salvador or Watling. The algæ collected were given to Professor Anthony Piccone for study, whose paper[8] is a useful contribution to phycogeography. Captain d'Albertis collected *Dasycladus occidentalis* Harv. and *Acetabularia crenulata* Lamour, in the interior salt lake of Guanahani. On July 21 he collected in the Atlantic, fifty miles off the mouth of the Delaware river, floating specimens of the following species: *Sargassum bacciferum* (Turn.) Ag., *S. vulgare* Ag., *S. filipendula* Ag., *Fucus vesiculosus* L., *Ascophyllum nodosum* (L.) Le Jol., *Jania rubens* (L.) Lamour (on the frond of *S. filipendula*). *Ascophyllum nodosum* was also collected in the Gulf Stream 150 miles S. E. of the Grand Banks of Newfoundland, and at 42° 6′ N. lat. and 46° 30′ W. long. Between New York and the Azores, d'Albertis collected *Sargassum Hystrix* J. Ag., *S. bacciferum* (Turn.) Ag., *S. cymosum* Ag., *Fucus vesiculosus* L., and *Ascophyllum nodosum* (L.) Le Jol.—DE TONI.

DR. G. KRAUS differs from the usually accepted view that calcium oxalate is a waste product of plant metabolism, and contends that it frequently plays the rôle of a reserve material.[9]

Finding by quantitative chemical analyses that the calcium oxalate present in roots of *Rumex obtusifolius* undergoes no noticeable diminution during the formation of its tall shoots in ordinary soil under usual conditions, he transplanted roots in early spring into pots containing in one case pure sand, and in the other case sand mixed with an abundance of calcium salts, and grew the plants in darkness to create the maximum demand on their reserves.

[7] That is, the true Guanahani, the first American island discovered by Columbus, not to be confounded with the false Guanahani or Cat, which is between Watling and Eleuthera.

[8] PICCONE, A.—Nota su alcune Alghe della Campagna del "Corsaro" in America. Atti della Società ligustica di Scienze naturali e geografiche **7**:—. 1896. [fasc. 4.] Genoa.

[9] DR. GREGOR KRAUS, Flora **83**: 54–73. 1897.

After two months analysis showed a marked loss of the calcium oxalate in the roots cultivated in pure sand, and but little decrease in the roots grown in the sand containing calcium. He concludes the oxalate here was drawn on to supply a demand for calcium.

The conduct of calcium oxalate in stems and branches was also investigated. In this connection analyses of barks with reference to the distribution of oxalate are of interest. The bark from trunks and branches of several trees gave uniformly a largely increasing amount of oxalate as one passes inward toward the cambium, the extremes in the oak being 4.96 per cent. in the outer cortex in the autumn, and 11.03 per cent. in the inner parts.

Analyses made at different times in the growing season showed that during the development of the buds the amount of calcium oxalate in the bark undergoes a marked decrease. The loss resulting from spring development was found to range between 12 and 42 per cent. of the amount present in the winter. This is accepted as evidence that calcium oxalate is made use of during the season of spring activity.

The solubility of calcium oxalate in various plant acids was tested and found to be considerable in concentrations varying from 0.1 to 0.001 per cent. Crystals examined after treatment presented a corroded appearance.

The author regards the water stream passing upward from the roots through the stem as the dissolving medium, and sees in the large calcium content often observed in the sap of trees a confirmation of this view.— RODNEY H. TRUE.

THE ECOLOGICAL RELATIONS of the underground systems of plants have been too little regarded, in spite of the fact that many of the most significant adaptations are of a subterranean nature. Rimbach's former studies[10] on underground stems and their methods of becoming deeply placed have been supplemented by a more comprehensive recent study.[11] In the meantime Areschoug has written a paper upon the same subject.[12] Areschoug has introduced the term "geophilous" plants, meaning those plants whose shoots persist in the soil, the antithetical term "aerophilous" denoting such plants as have aerial shoots. The geophyte condition is an adaptation against climatic extremes; annuals die when the dry or cold season advances, trees and shrubs protect themselves by lignification, while most perennial herbs

[10] Biologie der Pflanzen mit unterirdischem Sprosse. Ber. d. deutsch. bot. Ges. **13**: 141–155. 1895. Tieflage unterirdisch ausdauernder Pflanzen. Ber. d. deutsch. Bot. Ges. **14**: 164–168. 1896.

[11] Lebensweise der geophilen Pflanzen. Ber. d. deutsch. bot. Ges. **15**: 92–100 1897.

[12] Beiträge zur Biologie der geophilen Pflanzen. Acta Reg. Soc. Phys. Lund. T. 6. 1896. See Bot. Cent. **68**: 20–24. ——.

seek protection by ceasing aerial activities and remaining essentially dormant within the soil. Areschoug divides the geophytes into tufted perennials, rosette perennials, perennials with much branched base, bulb perennials, and rhizome perennials. The tufted and rosette types are not true geophytes, but are transitional forms. The third type sends up an aerial shoot the first season; this shoot dies down to the surface, and the next year branches from the base at several points; ultimately the basal parts are quite complex. Bulbous and rhizomatous plants represent the typical geophytes. Monocotyls have worked out better geophilous adaptations than have dicotyls. One of the important functions of geophilous plants is to store up a reserve food supply in the roots, stems or leaves. Plants with horizontal axes wander from year to year, more commonly in a straight line, though sometimes in a circle (orchids). Many plants become more deeply placed year by year. This burying is effected (1) by a downward growth of the stem, in which case the old stem parts are left behind; (2) by root contraction, in which case the plant is pulled down into the soil as a whole; or (3) by the intercalary growth of the petiole. Plants seem to have the power of self regulation, burying rapidly if put in shallow soil, slowly if put in deep soil. Rimbach considers this to be a matter of reciprocal action between leaves and roots; deep stems use up more energy in getting to the light and have a shorter period for assimilation, hence less energy can be expended in the work of burying deeper.—H. C. C.

THE ANNUAL REPORTS of a few of the Experiment Stations contain valuable botanical matter in addition to that issued through the bulletins. L. R. Jones, in the Vermont Report for 1895 (pp. 66–115), writes on potato blights, potato scab, oat smut, onion mildew, making and use of Bordeaux mixture, with many valuable original observations and deductions. W. C. Sturgis, in Connecticut Report for 1895 (pp. 166–190), writes on potato scab, onion smut, plum leaf curl, and notes on other diseases. S. M. Bain, in the Tennessee Report for 1896 (pp. 16–19), gives notes upon plant diseases observed within the state. B. D. Halsted, in the New Jersey Report for 1895 (pp. 247–361), and also in the Report for 1896 (pp. 287–429), records observations upon a large number of plant diseases, the fungi causing them, and on trials of fungicides, together with some other matters of botanical interest.—J. C. A.

MR. G. N. CALKINS finds tetrad formation and a reduction division in two ferns, *Pteris tremula* and *Adiantum cuneatum*.[13] The mitosis of the spore mother cells was studied, and the author finds that the chromosomes in both divisions behave in general as has been described by Häcker, Rückert, and vom Rath, for the maturation divisions in a number of animals. The reduction in the number of the chromosomes before the first division is a "pseudo-reduction," and in the second division we have probably only a transverse and no longitudinal splitting of the chromosomes.

[13] Bulletin of Torrey Bot. Club **24**. 1897.

The author uses largely the terminology of spermatogenesis in animals to describe the well known stages in spore development. The desirability of this innovation is perhaps questionable. We have come to use, to be sure, a common nomenclature for many stages in the vegetative mitosis of both plant and animal cells, but until the significance of the processes in the sporange and the ovary and testis are better understood it is perhaps best not to insist too much on the value of apparent analogies. Spore development and reduction of the chromatin are undoubtedly associated with alternation of generations in plants, and until it is settled whether a similar relation exists in animals a separate terminology is desirable. The author expresses much surprise that "such obvious structures as tetrads should have been hitherto overlooked in the plant reproductive cells." This is, however, merely a question of name, since he does not dispute the accuracy of the figures for the lilies as given by Farmer, Strasburger, and others. The term is certainly not very applicable to the figures in lilies, where the contraction of the chromosomes in the prophases does not go so far as to reduce them to almost spherical shape as in the ferns. This fact probably makes the lilies more favorable for the study of reduction than are the ferns. The existence of "tetrads" can hardly be regarded as settling the question of a reduction division in Weismann's sense. Mottier, in the latest paper on the lilies, admits that two interpretations of the figures are possible, and that the occurrence of a longitudinal splitting of the chromosomes in the second division is not absolutely excluded.—R. A. H.

THOSE INTERESTED in the physiology of the fungi will find record of a large number of experiments by Alfred Lendner upon some Mucorini and conidial forms of Ascomycetes in *Annales des Sciences Naturelles* (Bot.) VIII. 3: 1—64. 1897. His investigations were addressed to the question of the combined influences of light and the substratum upon the development of the fungi which were selected haphazard. The results have proved very variable not only in the two groups, but even in the same species, so that no general conclusions have been reached.—C. R. B.

H. TITTMANN has studied the formation and regeneration of periderm, epidermis, wax-coverings and cuticle in various plants.[14] His researches were conducted at Leipzig, under the direction of Professor Dr. Pfeffer.

The effect of increased pressure upon the formation of the periderm was investigated by means of gypsum bands surrounding young twigs of various woody dicots. This produces a retardation of cork development, but does not suppress it, as Newcombe had already shown,[15] the cork cells departing only slightly in number and form from those produced under normal conditions.

[14] Jarhrbücher f. wiss Botanik **30**: 116-154. 1897.

[15] BOT. GAZ. **19**: 149. 1894.

The regeneration of the periderm is not prevented by the checking of secondary thickening. In the open twigs from which the periderm was sliced replaced it from the cortical parenchyma, though the number of cells was not so great as the normal, except in *Sambucus nigra* in which they were more numerous. In a moist atmosphere the exposed cortical cells grew into long tubes, forming a callus, from which the periderm was produced.

No regeneration of epidermis was observed, but its removal was followed by the formation of cork or of callus and then cork.

Wax coverings were replaced in only three plants observed, *Ricinus communis*, *Rubus biflorus*, and *Macleya cordata*, and then only when the plants were still in vigorous growth. Several sedums and echeverias examined did not secrete wax after it had been removed. Light produced no effect upon this process, and moist air diminished, but did not entirely prevent it.

The removal of the cuticle could only be accomplished upon leaves with a very thick outer epidermal wall, such as the agaves and aloes possess. When sliced off it was reformed, even in the moist air, in which, however, it was thinner. Filaments of *Cladophora glomerata* were cut into pieces (which do not grow longer) and cultivated for four weeks. The transverse walls, now exposed, became covered with a cuticle. Typical water plants, like *Ceratophyllum demersum* and *Elodea Canadensis*, could not thicken the cuticle on exposure to air, so that it was impossible to cultivate them under new conditions. Even the submersed leaves of *Nuphar luteum* and *N. advena* could not live as floating leaves. On the contrary the water leaves of *Sagittaria sagittifolia* and *Hippuris vulgaris*, upon exposure to air, lived and thickened the cuticle strongly. Some land plants (*Mentha aquatica*, *Polygonum Hydropiper* and *Lysimachia nummularia*) easily adapted themselves to a submersed life, forming then only a very delicate cuticle as a result of diminished transpiration.

The delicate membrane covering the cells bordering upon the large intercellular spaces of many water plants (and some land plants also), designated as cuticle by Frank, reacts to increased transpiration, becoming partly lifted up from the cell walls into blisters or considerably thickened. Whatever its nature it is certainly not equivalent to the true cuticle.—C. R. B.

NEWS.

MR. ALFRED W. BENNETT, Lecturer on Botany at St. Thomas' Hospital, London, has been appointed editor of the *Journal of the Royal Microscopical Society*, to succeed Professor F. J. Bell.

AS WE ARE GOING to press the death of Dr. Julius Sachs, the eminent physiologist, is announced, having occurred at Würzburg, May 29. The GAZETTE hopes soon to publish a biographical sketch, prepared by Dr. Fritz Noll.

DR. J. N. ROSE has gone to Mexico for a summer of collecting. He left Washington for Guaymas the last of May, where he will spend some time with Dr. Palmer. Later at Mazatlan he will meet Mr. E. W. Nelson, and together they will cross the mountains into Durango and Jalisco.

SUNSTROKE is the name given to a physiological condition of the grape-vines observed in 1895 in California, and known in France as *folletage*. The leaves wither and drop off in hot weather, without apparant preliminary symptoms. The cause appears to be connected with the supply or movement of water in the plant, but no exact study has yet been devoted to the subject.

DR. EMILY GREGORY, Professor of Botany in Barnard College, died at her home in New York City, April 21. The name of Miss Gregory is a familiar one to botanists, both as author and teacher, and the announcement of her death, at the very height of her activity, occasions widespread sorrow. The GAZETTE is glad to be able to publish one of her last contributions, a review of Haberlandt's important *Physiologische Pflanzen-Anatomie.*

AT THE MEETING of the Academy of Science of St. Louis, held on the evening of May 3, 1897, Mr. H. von Schrenk spoke of the respiration of plants, with special reference to the modification of those growing with their roots submerged in water. The lecture was illustrated by a demonstration of the liberation of carbon dioxide in respiration from the roots of an ordinary flowering plant and freshly gathered fungi, and the more usual aerenchyma structures were made clear by the use of lantern slides.

GENERAL INDEX.

The most important classified entries will be found under Contributors, Geographical Distribution, Personals, and Reviews. Names of new species are printed in **boldface** type; synonyms in *Italics*.

A

Abies, firma 293; pectinata 292
Acrospermum album 370; compressum 370; corrugatum 370; foliicolum 370; fultum 370; graminum 370; Ravenelii 370; viridulum 370; urceolatum 367
Æcidia, Richards on development of 67
Æcidium elatinum 293
Agardh, J. G., work 63
Agardhinula 64
Agaricus, campestris 297; melleus 292
Agricultural Department, publications of U. S. 145; reorganization 200
Agrimonia, Bicknell on 141
Alabama flora, Mohr on 301
Alchemilla nivalis 7; **ocreata** 7
Algæ, of Belgium 71; of Corsaro expedition 477; culture methods 215: of Minnesota 95; polymorphism 62; new American 196; new U. S. 63; in solfatara 198; West and West on African 142, 301
Alisma, oosphere nucleus 41
Allen, T. F., work 141
Allin, Norra (& Macbride), work 65
Allocarya, Greene on 64
Amanita, caesaria 297; muscaria 297
Ambrosia trifida 194
Amentiferæ and Salix 169
Anderson, A. P., personal 71; "Abnorme Bildung von Harzbehältern" 292
Angiopteris evecta 21, 26, 27, 28, 227
Angiosperms, origin of 66
Antherozoids, in Zamia 458
Antipodals in Sagittaria 260
Apostaciaceæ, Ridley on 64
Arisaema tiphyllum 327
Arthur, J. C., 44, 57, 61, 66, 68, 296, 302, 381, 382, 383, 475, 479.
Ascobolus, Harper on 223
Ascomycetes, Harper on development of fruit 222; heteroecism 388
Asimina **Costaricensis** 2
Aspidium simulatum 64
Asplenium Bradleyi 394; *Ceratolepis* 251; montanum 394
Assimilation (see Photosyntax).
Aster, tardiflorus, Fernald on 142; diffusus 194
Astrophea 246, 247
Atkinson, Geo. F., 210, 372
Autran & Durand's "Hortus Boissierianus" 69

B

B. A. A. S., Toronto meeting 228
Bacteria, Pammel on gases produced by 61; Marshall on 296; toxic effect of dilute solutions, Paul and Krönig on 223
Bacteriology, prizes for research 232
Bailey, L. H., personal 228, 230; "Forcing book" 468; "Teachers' Leaflets for Nature Study" 144; "Survival of the Unlike" 144
Barnes, Charles R., 65, 130, 131, 134, 135, 140, 211, 214, 380, 385, 480
Barnes & Heald's "Keys to Mosses" 133
Bastin, Edson S., death 393
Batalin, A., death 226
Batrachospermum crouanianum 102
Beal, W. J., 51, 128, 204; "Grasses of N. A." 212
Begoniaceæ, DeCandolle on 64
Behrens' method for preserving fruits 145
Belgium, algal flora 71
Bennett, A. W., personal 482
Bessey, C. E., work 140
Bicknell, E. P., work 141, 301
Blasdale, W. C., work 142
Bokorny, Th., work 224
Bonnier, G., personal 392
Botrychium, species of 464; australe 287; *biternatum* 282; rutaceum 285; ternatum 282, 394; ternatum lunarioides 282; Virginianum 283; Virginianum, prothallus 391

Brainerd, Ezra, personal 230
Breyniastrum 2
Britton, E. G., work 141; N. L. 204; work 301
Bromus secalinus, germinating on ice 204
Buddleia, globosa 10; **megalocephala** 10
Buettneria Carthaginensis 240; **macrocarpa** 239
Burnap, C. E., 180
Burt, E. A., 63

C

Caeoma *Zeæ* 45
Cactaceae, monograph of 469
Caldwell, O. W., 42, 62, 298
Calea **Guatamalensis** 9; manicata 9; Robinson & Greenman on 65
Calcium oxalate, rôle of 477
California, opportunity for research at University of 75
Calosphace 249
Calostoma, notes on genus 180; Berkeleyi 188; cinnabarimum 181, 189; *cinnabarinum* 190; lutescens 187, 190; Ravenelii 190
Calyptranthes, rigida 245; **Tonduzii** 245
Campbell, D. H., work 139
Capparis **filipes** 2
Carbonic dioxide and growth 60
Cardamine, Greene on 141
Carex Fraseri, Holm on anatomy 297
Carelton's Uredineæ 227
Carica **dolichaula** 247
Caruel Teodoro, personal 392
Cassia **Guatemalensis** 6
Celloidin imbedding 393
Cell wall, Müller on 388
Central America, plants 1, 235
Centrosomes, of diatoms, Lauterborn on 299; of Lilium Philadelphicum 435
Centrospheres of Sagittaria 253, 257
Cestrum **dasyanthum** 11
Chaetochloa, Scribner on 301
Chaetophora, **calcarea** 97, 102; cornudamae 102; pellicula 102; tuberculosa 102
Chamaecyparis Lawsoniana 293
Chamaesenna 6
Chamberlain, C. J., 41, 59, 147, 298, 423
Chantransia pygmaea 102
Cheiranthus, Greene on 141
Chemotaxis 417
Cheney, L. S., 292, 388
Chicago, opportunity for research at University of 77
Chondromyces 205; **apiculatus** 405; aurantiacus 407, crocatus 406, 408; erectus 407; **gracilipes** 406
Chromosomes 439; of Lilium Philadelphicum 433
Christ, H., work 64
Chrysanthemum Leucanthemum 194
Chrysosplenium, alternifolium 274; alternifolium *tetrandrum* 275; Americanum 277; **Behringianum** 275; *glechomaefolium* 277; oppositifolium *Scouleri* 277; **Scouleri** 277; tetrandrum 275
Cibotium regale 27
Clathreæ, Burt on 63
Claytonia Virginica 116
Cleome pilosa **Costaricensis** 235
Clinostat, prize for 232
Coccochloris Orsiniana 199
Coelastrum **proboscideum** 196
Collomia, Coville on 302
Color in plants 466
Colubrina, **spinosa** 4; *spinosa* 242, 243
Columbia University, opportunities for research at 78
Combretum farinosum **phaenopetala** 7
Compositae, Franchet on 142; Klatt on 64
Conifers, fertilization and embryogeny 40
Contributors: Arthur, J. C. 44, 57, 61, 66, 68, 296, 302, 381, 382, 383, 475, 479; Atkinson, George F. 210, 372; Barnes, Charles R. 65, 130, 131, 134, 135, 140, 211, 214, 380, 385, 480; Beal, W. J. 51, 128, 204; Britton, N. L. 204; Burnap, C. E. 180; Burt, Edward A. 63; Caldwell, O. W. 42, 62, 298; Chamberlain, Chas. J. 41, 59, 147, 298, 423; Cheney, L. S. 292, 388; Cook, Mel. T. 43; Copeland, Edwin B. 303; Coulter, John G. 42, 58, 140, 220; Coulter, John M. 40, 60, 62, 64, 69, 132, 133, 134, 138, 139, 141, 210, 212, 213, 218, 219, 221, 296, 297, 301; 412; Coville, Frederick V. 206; Cowles, H. C. 478; Davenport, George E. 282; Davis, Bradley M. 67, 205, 216, 391; DeToni, J. B. 63, 209. 477; Dodge, Raynal 32; Fernow B. E. 219; Foerste, Aug. F. 460; Gregory, Emily 472; Groff, E. S. 468; Harper, R. A. 387, 388, 479; Henderson, L. F. 124; Hill, E. J. 121; Holm, Theodore 113; Humphrey, J. E. 50; MacDougal, D. T. 60, 142, 207, 291, 294, 297, 307; Merrell, W. D. 42, 43; Mott, F. T. 466; Olson, Mary E. 367; Penhallow, D. P. 15; Ramaley, Francis 224; Robertson, Charles 288; Robinson, B. L. 135; Russell, H. L 223; Schaffner, J. H. 40, 66, 252, 299.

Contributors: 430; Smith, John Donnell 1, 235; Swingle, W. T. 278; Thaxter, Roland 63, 222, 389, 395; Tilden, Josephine E. 95; Trelease, W. 470; True, R. H. 477; Uline, E. B. 469; Underwood, Lucien M. 464; Waugh, F. A. 193; Webber, H. J. 453; Wittrock, Veit B. 196; Zoethout, W. D. 467
Cook, Mel. T. 43
Copeland, E. B. 303; personal 393; "Einfluss von Licht und Tempertur auf Turgor" 134
Cordyline terminalis 26
Coreopsis tinctoria 194
Cormonema Mexicana 243; Nelsoni 243; **ovalifolium** 242; spinosum 243
Cornell University, opportunities for research at 80
Corn, Hitchcock on smut 296; common ustilago of 44
Cortex of Myeloxylon 17
Costus **podocephalus** 250
Coulter, J. G. 42, 58, 140, 220
Coulter, J. M. 40, 60, 62, 64, 69, 132, 133, 134, 138, 139, 141, 210, 212, 213, 218, 219, 221, 296, 297, 301, 412
Coville, F. V. 206; work 302
Cowles, H. C. 478
Crataegus Vailiae, Britton on 301
Crepis, Greene on 64
Cucubalus inflatus 137
Cummings, Williams & Seymour's "Lichenes Boreali-Americani" 218
Currant canes, Durand on disease of 296
Cyanophyceæ, Setchell on 142
Cycadaceæ 27, 30; anatomy of stem 138; fossil 66
Cycas revoluta 27, 28; Ikeno on spermatozoids 221
Cyrtandraceæ, Ridley on 64
Cystobacter, aureus 408; *erectus* 407; fuscus 408; simplex 408
Cytoplasmic radiations 445

D

Dalea, Vail on 301
Dana's "Plants and their Children" 130
Dangéard (Léger &), work 389
Daucus carota 296
Davenport, G. E. 282; "experimental morphology" 467; work 64
Davis, Bradley M. 67, 205, 216, 391
De Candolle, C., work 64
Delphinium, Leiberg on 302
Dentaria, Greene on 141
Derschau's "Einfluss von Kontakt und Zug auf rankende Blattstiele" 142
De Toni, J. B. 63, 209, 477; personal 227
Diatoms, Ckalkley on photosyntax 297; Klebahn on 387; Lauterborn on centrosomes 299
D'Hubert, E., on embryo sac 59
Dichothrix **calcarea** 100
Dicliptera **sciadephora** 13
Dioon edule 24, 27, 28
Diplostephium, **corymbosum** 8; **paniculatum** 8
Discomycete, a new, on Selaginella 367
Diseases: Currant canes 296; forcing house and garden plants 296; forest trees 232; grape 57; Larix Europaea 57; oedema of Salix nigra 234; peach 296; Pinus montana 58
Distribution of plants 233
Dodge, Raynal 32; "Ferns of New England" 134
Drude, O., work 62
Durand, E. J., work 296

E

Ecology, Colorado plants 232; protection of pollen 65
Editors, foreign associate 48
Ellis & Everhart, work 65
Embryo, of Coniferae 40; of Lilium Philadelphicum 418; of Sagittaria 262; of Salix 160
Embryo-sac, of Lilium Philadelphicum 413; of Salix 154; of succulents 59
Endosperm, Lilium Philadelphicum 420; of Sagittaria 260; of succulents 59
Endophytes, Janse on root 298
Engler's "Geographische Verbreitung der Rutaceen" 220; & Prantl's "Natürlichen Pflanzenfamilien" 146
Epidermis, formation and regeneration of 480
Eriksson, Jacob, personal 392
Errera & Laurent's "Planches de Physiologie Végétale" 211
Erysimum, Greene on 141
Erysiphe communis, Harper on 222
Erythronium albidum 288
Erythroxylum, amplum 240; **Costaricense** 240; laurinum 240
Escallonia, myrtilloides 244; **Poasana** 243
Esenbeckia, Acapulcensis 242; Berlandieri 242; **litoralis** 242
Ettingshausen, C. von, death 226, 392
Eugenia **Guatemalensis** 245
Eupatorieæ, Hieronymus on 302

F

Farlow's "Cryptogamic Botany in Harvard University" 144
Fawcett, William, work 142
Fermentation 61
Fernald, M. L., work 142
Fernow, B. E. 219
Ferns, Christ on 64; reduction division in 479
Fermentation, action of yeast cell 475.
Fertilization 253; in conifers 40; of Lilium Philidelphicum 417; in Salix 158; in Sagittaria 255
Flowers and insects, Robertson on 141; organogeny of Salix 149
Foerste, Aug. F. 460
Food of green plants, Bokorny on 224
Forcing house, diseases of plants in 296
Forest, reservations 306; trees, diseases of 232
Forestry monographs 55
Fossil plants 15–66
Fournieria, Scribner on 301
Franchet, M. A., work 142
Freesia refracta alba 195
Fruits, preserving juicy 145
Fungi, Ellis & Everhart on tropical 65; Lloyd's photogravures of 394; Lloyd's museum of 394; physiology of 480; Underwood on 301
Fungus *pulverulentus* 189
Fusarium *Flichianum* 58

G

Gagea lutea 117, 118; minima 117
Gametophyte of Sagittaria 253; of Salix 150, 154
Gardens, Botanical 51; Berlin 392; Buitenzorg 71; Peradeniya 72, 393; Kew 226; Missouri 144, 230; New York 229
Geaster, relation with Calostoma 186
Geographical distribution 1, 43, 63, 65, 71, 95, 124, 142, 196, 233, 235, 296, 301, 477
Geophilous plants 478
Geothallus, Campbell on 139
Geotropism of roots 325
Geranium, Britton on a new 301
Germination, influence of electric current on 302
Ginkgo biloba, Hirase on spermatozoids 221
Goff's "Principles of Plant Culture" 382
Grain, prize for memoir on 306
Grape, disease 57
Grasses, Nash on new species 301; Pammel & Scribner on 142; Scribner on 301; Scribner & Smith on 301; of Dakota, Williams on 301
Gray's Synoptical Flora 228; collection of letters 144
Gray, Mrs. Asa, personal 144
Greene, E. L., work 64, 141
Greenman, J. M., work 65
Gregory, Emily 472; death of 482
Groff, E. S. 468
Growth, influence of electric current on 302
Guatemala, plants 1, 235
Guatteria dolichopoda 2; **nigrescens** 2; **oliviformis** 1
Guignard, L., personal 71
Gum, exudation from grape 57; canals of Angiopteris 29
Gymnogramme Ceratolepis **Atirrensis** 251
Gyropodium *coccineum* 187, 189

H

Haberlandt's "Physiologische Pflanzenanatomie" 472
Hair balls from Opuntia 233
Halsted, B. D., personal 72
Hansgirg, A., work 65, 298
Harper, R. A. 387, 388, 479; work 222
Harshberger's "Botanists of Philadelphia" 227
Harvard University, opportunities for research at 81
Harvey, F. L., work 296
Hazlinszky, F. A., death 144
Heliocarpus *Americanus forma* 240; polyandrus **nodiflorus** 240
Heliotropism 315
Heller, A. A., work 302
Henderson, L. F. 124
Hendersonia montana 58
Henry, Augustine, work 145
Herbarium, Biltmore 226; Jas. Lloyd 228; Joor 227; Schott 228
Heteroecism in Ascomycetes 389
Hieracium aurantiacum 296
Hiern's "Catalogue of Welwitsch's African Plants" 210
Hieronymus, G., work 302
Hill, E. J. 121
Hirase, S., work 221
Hitchcock, A. S., work 296
Holm, Theodore 113; work 297, 301
Hopi, plants used by 306
Honey dew, Bonnier on 140
Horticulture, Encylopedia of America 230

Howe, M. A., personal 71
Humphrey, J. E. 50
Hypostomum Flichianum 58
Hypoxis, erecta, nomenclature of 113, 206; *sessilis* 116

I

Ichthyocercus, West & West on 301
Idaho, Leiberg on flora 296; new Isoetes 124
Ikeno, S., work 221
Illinois, opportunities for research at University 83
Impatiens, *fulva* 242; **Turrialbana** 241
Indiana, Proceedings Academy of Science 68
Iowa, mesophytes and xerophytes of 233; Pammel on flora of 142; Pammel's plants of 226; puff balls 65
Ipomoea **leucotricha** 10; sericophylla 11
Irish, H. C., work 392
Irritability of roots and shoots 313
Isoetes, Bolanderi 124; **Eatoni** 36, 134; echinospora 33, 38; Engelmanni 32; Engelmanni valida 34; foveolata 134; Montezumae 394; Nuttallii 124; **Underwoodi** 124
Ixophorus, Scribner on 301

J

James, Joseph F., death 393
Janse, J. M., work 298
Jacaratia 248
Jeffrey, E. C., work 391
Johns Hopkins University, opportunities for research at 84
Johnson, Lorenzo N., death 229
Jones, L. R., personal 230
Jost, L., work 297
Juranyi, L., death 392
Jussieua, **geminiflora** 246; latifolia 246

K

Kentia Fosterana 26
Kew publications 226
Kienitz-Gerloff, personal 144
Kinney, A. S., work 302
Klatt, F. W., work 64
Klebahn, H., work 387
Klebs' "Bedingungen der Fortpflanzung" 214
Klercker, J. af, work 62
Koch, R., personal 72
Koehne, E., work 142
Krabbe, G., work 303

L

Laboulbeniaceæ, Thaxter on 216
Laboratory, material for 372; tropical 47, 50, 54, 126, 129, 202, 207, 291
Larix Europaea, disease of leaves 57; Japonica 294
Lauterborn, R., work 299
Leaves, curious modifications of 460; growth and chlorophyll function 60
Lechea, Bicknell on 301
Ledum palustre 388
Léger & Dangéard, work 389
Leiberg, J. B., work 302; "Botanical Survey Cœur d'Alene Mts." 296
Leland Stanford Junior University, opportunities for research at 85
Lepachys columnaris 195
Leucelene, Greene on 142
Lichens, epiphytic 72
Lilium Philadelphicum, embryo of 418; embryo sac of 413; endosperm of 420; fertilization in 417; life history of 412; macrospore nucleus of 430; pollen grain of 423
Lippia substrigosa **oxyphyllaria** 249
Lloyd, museum of fungi 394; photogravures of fungi 394
Lloyd, James, death 228
Loasa, argemonoides 8; **bipinnata** 7; chelidonifolia 7; **speciosa** 8
Lodeman, E. G., death 71
Loefgrenia 196; **anomola** 197
Lycoperdaceæ, Macbride & Allin on 65
Lycoperdon *calostoma* 189; *heterogeneum* 187, 189; *Zeæ* 45
Lyngbya, martensiana 101; martensiana **calcarea** 101; Lagerheimii 101; **nana** 101; ochracea 101; purpurea 101; rivularium 101

M

Macbride & Allin, work 65; & Smith, work 65
MacDougal, D. T., 60, 142, 207, 291, 294, 297, 307
MacMillan, Conway, personal 392, 393
Macoun's "Contributions from Herbarium Geological Survey of Canada" 132
Macrospore, in Sagittaria 254; in Salix 152, 154
Macrozamia, Worsdell on anatomy 138
Magnus, P., personal 227
Maize, common ustilago of 44
Malvaviscus **Palmanus** 238
Markea *neurantha* 12
Marshall, C. E., work 296

Massee's "Redescriptions of Berkeley's Types of Fungi" 133
Mauria, Biringo 5; glauca 5
Medullosa 15; elegans 22, 23; Leuckarti 25; porosa, 22; stellata 22
Melandryum, Williams on 136
Melastomaceæ, Fawcett on 142
Meria Laricis 57
Merinthopodium 11; **neuranthum** 12
Merrell, W. D., 42, 43
Metric system in botany 126, 204
Mexico, plants from 146; new species from 65
Mez, Carl, personal 226
Michigan, botanical papers at Academy of Science 394; opportunities for research at University of 85; Wild Flower Co. 393
Micronucleoli 433
Microspores of Sagittaria 253; of Salix 150
Mikania, Robinson & Greenman on 65
Millspaugh's "Flora of Yucatan" 132
Mimosa, Jost on 297
Minnesota, algæ 95; opportunities for research at University of 86
Missouri Botanical Garden 231; opportunities for research at 231
Mitremyces *cinnabarinum* 188; *heterogeneus* 187, 189; *lutescens* 188, 189, 190; *Ravenelii*, 189, 190
Möbius' "Lehre von der Fortpflanzung der Gewächse" 385
Mohr, Charles, work 301; "Timber Pines of the Southern United States" 55
Morphology, experimental 467
Mosses, Britton on Bolivian 141
Motor zones 316, 324
Mott, F. T. 466
Mounting plants for lecture demonstrations 128
Movements of roots 307
Mucilage canals of Angiopteris 29
Mucorineæ, cytology 389
Mucor rigidus 390; rubescens 390
Müller, Baron F. von, personal 227
Müller, Carl, work 388
Müller, Carl, personal 144
Mushrooms, Underwood on 297
Mutinus caninus 63
Mycorhiza, Janse on 298
Myelopteris **Topekensis** 15, 227
Myeloxylon 15
Myrosma **Guapilesense** 251
Myriostoma coliforme 43
Myrtus 245
Myxobacteriaceæ 205, 395
Myxobotrys *variabilis* 205
Myxococcus, **cirrhosus** 409; coralloides 408; **cruentus** 409; **stipitatus** 408
Myxomycetes, Macbride & Smith on 65

N

Naples Zoological Station, opportunities for research at 278
Nash, G. V., work 301
Nawaschin, S., work 388
Nebraska, botanical seminar at University of 229; opportunities for research at University of 89
Nectaries, septal 470
Nectria cinnabarina 296
Nestler, A., personal 227
Newcombe, F. C., personal 394
New York Botanical Garden 229; plants 43
Nicaragua, fungi 65; Myxomycetes 65
Nichols, Mary A., personal 71
Nitella, Allen on 141
Noll, F., personal 392; "Sinnesleben der Pflanze" 295
Nomenclature 44, 113, 290
Nordstedt's "Index Desmidiacearum" 209, 210
Norton, J. B. S., work 66
Nucleoli 443; migration of 431
Nucleus, phenomena of "reduction division" 431
Nutrition of green plants, Bokorny on 224

O

Œdema of Salix nigra 234
Œdogonium, echinospermum 197; Landsboroughi **robustum** 197; **Lindmanianum** 197; **Wittrockianum** 198
Oldenlandia, Mohr on 301
Olson, Mary E., 367
Opuntia, hair balls from 233
Orchidiceæ, Ridley on 64
Oreastrum, Greene on 142
Oreocarya, Greene on 64
Ornithogalum, *hirsutum* 206, 113; luteum 114; minimum 114; umbellatum 117
Osmosis, influence of temperature on 303
Osmunda *biternata* 282
Oxalis *pilosissima* 241, **vulcanicola** 241

P

Palmacites 15; carbonigenus 22; leptoxylon 22
Palmer, T. C., work 297
Pammel, L. H., work 142, 232; and wife work 61

Panicums, Holm on 301
Parenchyma of Myeloxylon 17
Parosela, Vail on 301
Passiflora, **pediculata** 247; **Pittieri** 246
Paul & Krönig, work 201, 223
Pavonia, Bahiensis 238; **oxyphyllaria** 237
Peach, Price on diseases of 296
Peck's "Report of the Botanist" 384
Peirce, G. J., personal 393
Penhallow, D. P. 15
Periderm, formation and regeneration of 480
Perithecium, Harper on development 222
Personals, Anderson, A. P. 71; Bailey, L. H. 228, 230; Bastin, E. S. 393; Batalin, A. 226; Bennett, A. W. 482; Bonnier, G. 392; Brainerd, E. 230; Caruel, T. 392; Copeland, E. B. 393; DeToni, J. B. 227; Eriksson, J., 392; Ettingshausen, C. von 226, 392; Gray, Mrs. Asa 144; Gregory, Emily 482; Guignard, L. 71; Halsted, B. D. 72; Hazlinszky, F. A. 144; Howe, M. A. 71; James, J. F. 393; Johnson, L. M. 229; Jones, L. R. 230; Juranyi, L. 392; Kienitz-Gerloff 144; Koch 72; Lloyd, C. G. 394; Lloyd, J. 228; Lodeman, E. G. 71; MacMillan, C. 392, 393; Magnus, P. 227; Mez, Carl 226; Müller, F. von 227; Müller, C. 144; Nestler, A. 227; Newcombe, F. C. 394; Nichols, Mary A. 71; Noll, F. 392; Peirce, G. J. 393; Pringle, C. G. 71, 230; Rose, J. N. 482; Rothert, W. 226; Saccardo, Fr. 71; Sachs, Dr. J. 482; Schrenk, H. von 72; Setchell, W. A. 226; Sheldon, E. P. 392; Spalding, V. M. 394; Stoneman, B. 226; Taubert, P. 227; Trimen, H. 144; Ville, G. 392; Ward, H. M. 228; Wheeler, C. F. 394; Willis, J. C. 72; Wooton, E. O. 71.
Pestalozzia, Hartigii 293
Phalleæ, Burt on 63
Phaseolus vulgaris 327; native country 226
Phenology, Irish on 392
Philadelphus, Koehne on 142
Phoenix dactylifera 26, 328
Phoma abietina 292
Photosyntax, demonstration in diatoms 297; relation of stomata to 58
Physiology, equipment for 47
Phyton, value of 377
Picea excelsa 293; embryo 42
Pilaira Cesatii 408
Pilea *sp.* 14
Pilinia *diluta* 102
Pinus, Banksiana, sperm nuclei 40, embryo 42; Laricio, embryo 42, nuclei 41, pollen tube 42: montana, fungus on leaves 58; Strobus 294; sylvestris 292; nuclei 41
Pisum sativum 327
Pittonia 10
Pleonectria berolinensis 296
Pollen, Hansgirg on 298, 65; of Salix 150, 158; of Lilium Philadelphicum 423
Polycephalum *aurantiacum* 407
Polymorphism of algae, Klercker on 62
Populus, microspores of monilifera 151; tremuloides 169
Porophyllum, Robinson & Greenman on 65
Potato, Watson on 297
Potentilla, Rydberg on 64, 301
Price, R. H., work 296
Primula Chinensis 195
Pringle, C. G., personal 71, 230
Proceedings Indiana Academy of Science 68
Prothallus of Botrychium 391
Protophyta, Bessey on classification of 140
Prunus Gravesii, Small on 301
Psephotaxus, West & West on 301
Pseudohelotium granulosellum 96
Psidium, basanthus 244; **savannarum** 244
Puff balls, Macbride & Allin on 65; Underwood on 297
Purdue University, opportunities for research at 90
Pyxispora, West & West on 301

Q

Quraribea, **platyphylla** 239; turbinata 239
Quillwort, new 32, 124, 134

R

Rachiopteris Williamsoni 21, 26, 29, 30
Ramaley, Francis 224
Ráthay, E., work 57
Reaction times in roots 323
Reduction division, in ferns 479; in Lilium 430
Rendle, A. B., work 142
Research, facilities for 73, 231, 278, 393; neglect of American 201, 205
Resin, canals of Angiopteris, see Gum; ducts, Anderson on 292

Respiration, intramolecular 234; of plants with submerged roots 482
Reviews: Anderson's "Abnorme Bildung von Harzbehältern" 292; Astran & Durand's "Hortus Boissierianus" 69; Bailey's "Forcing book" 468; "Teachers' Leaflets on Nature Study" 144; Barnes & Heald's "Keys to Mosses" 133; Beal's "Grasses of North America" 212; Copeland's "Einfluss von Licht und Temperatur auf Turgor" 134; Cummings, Williams & Seymour's "Lichenes Boreali Americani" 218; Dana's "Plants and their Children" 130; Davenport's "Experimental morphology" 467; Derschau's "Einfluss von Kontakt und Zug auf rankende Blattstiele" 142; Dodge's "Ferns of New England" 134; Engler's "Geographische Verbreitung der Rutaceen" 220; Errera & Laurent's "Planches de Physiologie Végétale" 211; Farlow's "Cryptogamic Botany in Harvard University" 144; Goff's "Principles of Plant Culture" 382; Haberlandt's Physiologische Pflanzenanatomie" 472; Hiern's "Catalogue of Welwitsch's African Plants" 210; Klebs' Bedingungen der Fortpflanzung" 214; Leiberg's "Botanical Survey of Coeur d'Alène Mts." 296; Macoun's "Contributions from Herbarium Geological Survey of Canada" 132; Massee's "Redescriptions of Berkeley's Types of Fungi" 133; Millspaugh's "Flora of Yucatan" 132; Möbius' "Lehre von der Fortpflanzung der Gewächse" 385; Mohr's "Timber Pines of Southern United States" 55; Noll's "Sinnesleben der Pflanze" 295; Nordstedt's "Index Desmidiacearum" 209, 210; Peck's "Report of the Botanist" 384; "Proceedings Indiana Academy of Sciences" 68; Robinson & Schrenk "Notes on Flora of Newfoundland" 69; Rothrock & Shunk's "Report Pennsylvania Forestry Commission" 219; Saccardo's "Index to Sylloge Fungorum" 381; Schimmel & Co.'s "Semi-annual Report" 135; Schniewand-Thies' "Septal nectaries" 470; Schumann's "Monographia Cactacearum" 469; Strasburger's "Botanisches Practicum" 380; Sudworth's "Nomenclature of Arborescent Flora of United States" 219; Thaxter's "Laboulbeniaceæ" 216; Tubeuf's "Diseases of Plants" 383; Uline's "Dioscoreæ Mexicanæ et Centrali Americanæ" 132; Willis' "Manual and Dictionary of Flowering Plants" 213; Wittrock's "Pansies" 69; Wright's "Organic Drugs of United States" 144; Zimmermann's "Morphologie und Physiologie des pflanzlichen Zellkernes" 131
Rhopalodia gibba 387
Ribes, Heller on 302
Richards, H. M., work 67
Ridley, Henry, work 64
Robertson, Charles 288; work 141
Robinson, B. L. 135; work 65; & Schrenk's "Notes on Flora of Newfoundland" 69
Rolfe, R. Allen, work 64
Roots, curvature of 307
Rose, J. N., personal 482
Rothert W., personal 226
Rothrock & Shunk's "Report Pennsylvania Forestry Commission" 219
Rourea **Suerrensis** 5
Rubus Guyanensis **vulcanicolus** 243
Rush, W. H., work 234
Russell, H. L. 223
Rydberg, P., work 64, 301

S

Saccardo, Fr., death 71
Saccardo's "Sylloge Fungorum, Index" 381
Sachs, Dr. J., death of 482
Sagittaria, Mohr on 301; variabilis, life history of 252
Salix, life history of 147; nigra, œdema of 234
Salvia, affinis 13; **monochila** 13; nervata 14; **Pansamalensis** 249; **phænostemma** 13
Sambucus, Leiberg on 302
Sanguinaria Canadensis 288
Saranthe 251
Saurauia, **Costaricensis** 236; Lehmanii 237; **Pittieri** 237; scabrida 237; strigillosa 237; veraguensis 237
Schaffner, J. H. 40, 66, 252, 299, 430
Schellenberg, H. C., work 58
Schimmell & Co.'s "Semi-annual Report" 135
Schizothrix Friesii 103; penicillata 104; rubella 103; **rupicola** 103
Schniewand-Thies' "Septal nectaries" 470
Schœnocrambe, Greene on 141
Schrenk, H. von, personal 72; work 234

Schumann's "Monographia Cactaclarum" 469
Scientific department, a national 200
Sclerenchyma of cycads and ferns 30; of Myelopteris 18
Scleroderma *calostoma* 187, 189
Sclerotinia, aucupariae 389; heteroica 388; *ledi* 388; megalospora 388; padi 389
Scribner, F. L., work 142, 301
Secretion organs of, in Angiopteris 28; in cycads 28; in Myelopteris 20
Seeds, care of 296; crests and ants 288; dissemination by wind 233
Selaginella rupestris, parasite on 367
Selby, A. D., work 296
Sensory zone of roots 318
Setchell, W. A., personal 226; work 142
Sheldon, E. P., personal 392
Silene, Williams on 62, 135; acaulis 138; Armeria 137; Behrii 137; campanulata 137; *Cucubalus* 137; Douglasii 137; Hallii 137; inflatus 137; Menziesti 138; monantha 137; *purpurata* 137; repens 137; Scouleri 137, 138; *veracunda* 137
Siphocampylus **discolor** 248; fœtidus 248; glandulosus 249; **roseus** 249
Sisyrinchium, Rendle on 142
Small, J. K., work 301
Smith College, opportunities for research at 92
Smith, C. L. (Macbride &), work 65
Smith, John Donnell, 1, 235
Smith, Jared G., work 301
Smut, Hitchcock on corn 296; nomenclature of corn 44
Soap trees of China, Henry on 145
Solanum rostratum 296
Sphaerotheca Castagnei, Harper on 222
Spalding, V. M., personal 394
Spermatozoids in Cycas and Ginkgo 221
Spindle threads 445
Spiraea filipendula 388
Spirogyra **Malmeana** 198
Stellaria, **Irazuensis** 236; micrantha 236
Stenzella 15
Stereosanthus, Fawcett on 142
Stevia, Hieronymus on 302
Stigeoclonium flagelliferum 102
Stigmatella *aurantiaca* 407
Stillum *rhytidospora* 407
Stimuli, for roots 318
Stomata, Schellenberg on 58
Stoneman, Bertha, personal 226
Strasburger's "Botanisches Practicum" 380
Strasburger, Noll Schenck & Schimper's "Lehrbuch der Botanik" 226
Succulents, embryo sac of 59
Sudworth's "Nomenclature of arborescent flora of U. S." 219
Sunstroke of grapevines 482
Surirella calcarata 300
Suspensor, in Lilium Philadelphicum 419; in Salix 160; in Sagittaria 262
Swingle, W. T. 278

T

Tannin sacs of Angiopteris 29
Taubert, P., death 227
Taxonomy 62, 64, 141, 290, 301; and variation 193
Taxus baccata, pollen tube 43
Temnogametum, West & West on 301
Temperature, influence on osmosis 303
Teratology 165
Thaspium aureum 121
Thaxter, Roland 63, 222, 389, 395; "Laboulbeniaceæ" 216
Tilden, Josephine E. 95
Tobinia 4
Tournefortia cymosa 10; **Nelsoni** 10
Transpiration, relation of stomata 58
Trécul, biography of 71
Trelease, William, work 233; 470
Tridax, Robinson & Greenman on 65
Trifolium, Blasdale on 142
Trigonia **thyrsifera** 3
Trillium recurvatum 288
Trimen, Henry, personal 144
True, R. H. 477
Tubercularia vulgaris 296
Tubeuf's "Diseases of Plants" 383
Tulostoma, relation to Calostoma 185
Turgor, temperature and 303

U

Uline, E. B. 469
Uline's "Dioscoreæ Mexic. et Centr. Amer." 132
Umbelliferae, Drude on 62
Underwood, L. M., work 297, 301; 464
Uredineæ Americanæ exsiccatæ 227
Uredo *Maydis* 45; *segetum* 45
Urera simplex 14; **Tuerckheimii** 14
Uroglena Americana 105; radiata 105, 107; volvox 105, 106, 107, 110, 111
Ustilagineæ of Kansas, Norton on 66
Ustilago, Hitchcock on 296; Zeæ 45; *Zeæ-Mays* 45
Uvularia grandiflora 288

V

Vaccinium uliginosum 388
Vail, Anna M., work 301
Vanilla, Rolfe on 64
Variation, significance in taxonomy 193
Vascular bundles of Angiopteris 28; of cycads 28; of Myelopteris 18
Verbesina Fraseri **Nelsoni** 9
Vermont Botanical Club 230
Vernonieæ, Hieronymus on 302
Vestal, George, work 296
Vicia Faba 329
Ville, Georges, death 392
Viola, blanda 54; cucullata 53; obliqua 53; rotundifolia 54; tricolor 69
Violets, Britton on new 301; Greene on acaulescent 53, 141
Volvox globator 106
Vuillemin, work 57

W

Waltheria **rhombifolia** 3
Ward, H. Marshall, personal 228
Ward, Lester F., work 66
Watson, T. L., work 297
Waugh, F. A. 193
Webber, H. J. 453
Weeds, troublesome 296
West, W. & G. S., work 142, 301
Wheeler, C. F., personal 394
Williams, F. N., work 62, 135
Williams, Thomas A., work 301
Willis, J. C., personal, 72; "Manual and Dictionary of Flowering Plants" 213
Wisconsin, opportunities for research at University of 93
Wittrock, Veit B. 196; "Pansies" 69
Wooton, E. O., personal 71
Woronin, M., work 388
Worsdell, W. C., work 138
Wright's "Organic Drugs of the U. S." 144

X

Xanthoxylum: see Zanthoxylum
Xerophytes of Iowa 233
Xylosma Benthami 235; intermedium 235; **oligandrum** 235

Y

Yeast cell, action in alcoholic fermentation 475

Z

Zamia, antherozoids in 458; peculiar structures of pollen tube 453
Zamia integrifolia 27, 28
Zanthoxylum Pringlei 4; **procerum** 4
Zea Mais 44, 296, 327
Zimmermann's "Morphologie und Physiologie des pflanzlichen Zellkernes" 131
Zinnia, Robinson & Greenman on 65
Zizia aurea 121
Zoethout, W. D. 467
Zukal, Hugo, work 205